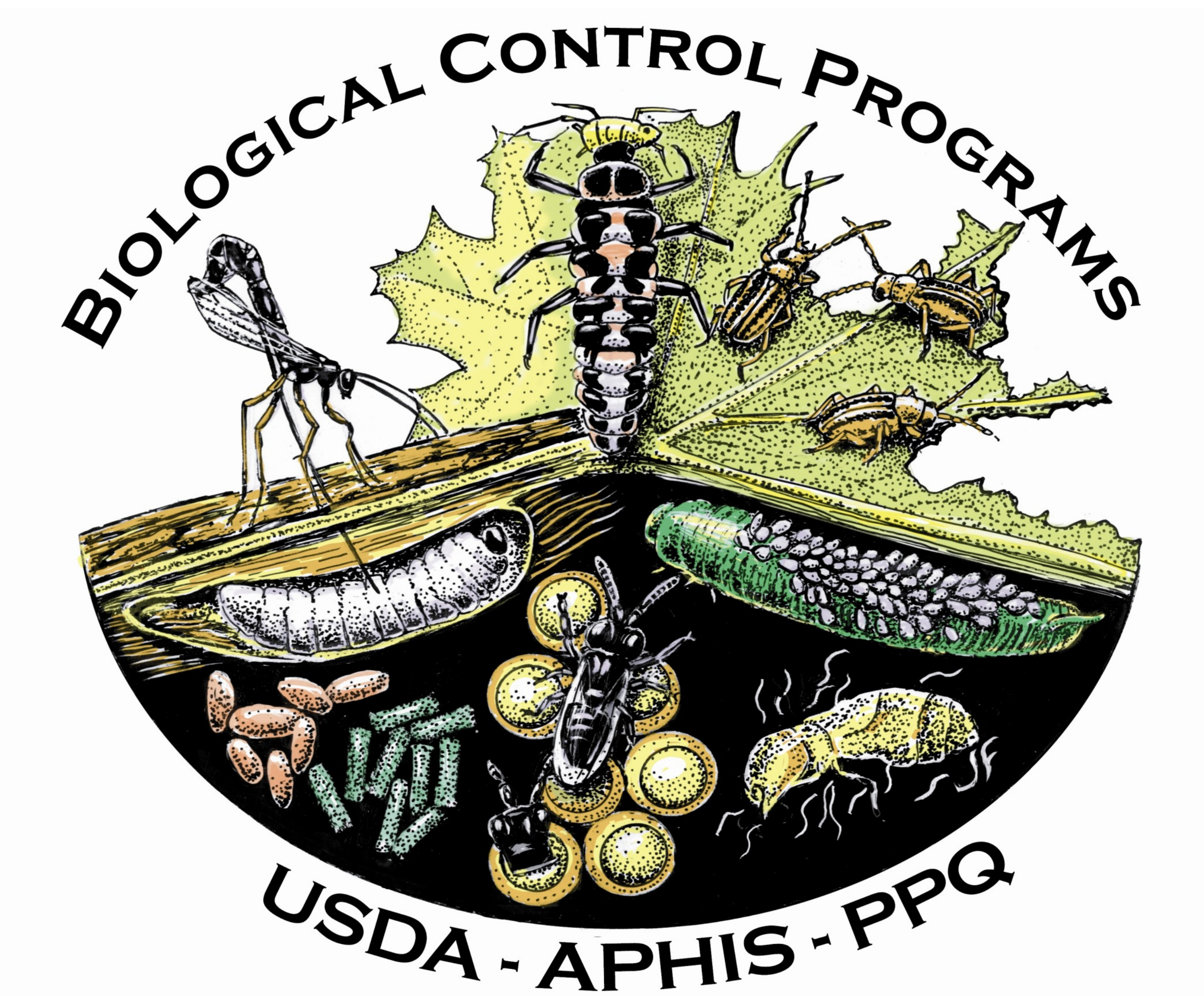
CPHST
BIOLOGICAL CONTROL UNIT



2010 ANNUAL REPORT

Cover Image Artist: Joel Floyd (PPQ NIS)

CPHST Biological Control Unit

2010 Annual Report

U. S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection & Quarantine
Center for Plant Health Science & Technology

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SUMMARY OF ACCOMPLISHENTS

In 2010, CPHST employed 18 scientists that focused part or all of their time developing biological control technologies to help mitigate the impacts of introduced, invasive insect pests, weeds, and plant pathogens. We received $3,485,000 of Biocontrol Line Item funding. The majority of these funds (ca. 88%) were used to support CPHST scientists directly working on biological control projects. The remaining 12% was used for equipment, supplies and cooperative agreements.

CPHST scientists provide technical oversight and expertise to programs to ensure that scientific knowledge gaps are identified and addressed, and that developed technologies are transferred to stakeholders as quickly as possible. Our scientists provide support in the discovery and evaluation of new biological control agents offshore and domestically; develop cost effective rearing and monitoring systems for approved biological control agents and their hosts; provide permitted biological control agents collected from established field insectaries and laboratory colonies to PPQ project managers and other project cooperators for redistribution; ensure the safety of biological control agents through on-going post-release monitoring; and develop educational and programmatic materials for use by PPQ and other collaborators.

WEEDS:

Canada Thistle & Hound's-Tongue: CPHST FCL continued to assess the distribution and impact of accidentally-introduced and native arthropods attacking the exotic weeds Canada thistle and hound's-tongue. The mite *Aceria anthocoptes* and the lace bug *Corythucha distincta* were shown to have a fairly broad host range that includes many native thistles, likely precluding their use as biocontrol agents. FCL initiated a survey of the root weevil *Mogulones cruciger* in WA and ID in 2010, through a grant provided to the University of Idaho. The UI survey will document the spread and non-target plant utilization by the weevil, which has migrated into the US from Canada.

Dalmation Toadflax & Field Bindweed: CPHST FCL assisted State Plant Health Directors and State Departments of Agriculture in the collection, distribution, and evaluation of the permitted and established weed biological control agents *M. janthinus* for Dalmatian toadflax control and the gall mite *Aceria malherbae* against field bindweed in western states.

Invasive Weeds: CPHST Ft. Collins Laboratory (FCL) renewed a cooperative agreement with CABI Europe-Switzerland through FAS for foreign exploration and pre-release host range testing of insect biological control agents attacking garlic mustard, yellow toadflax, hoary cress, hound's-tongue, Russian knapweed, dyer's woad, perennial pepperweed, field bindweed, hawkweeds, and Canada thistle. These weeds were identified as high priority targets by Eastern and Western Regions after canvassing State Plant Health Directors and other entities in each state. CPHST is partnering in its support for these weed biological control targets with a wide variety of partners and funding consortia, including USDA-ARS, USDA Forest Service, Montana State University, California Department of Food and Agriculture, Wyoming Weed and Pest Districts, and Agriculture and Agri-Foods Canada. In 2010, FCL collected seeds of native plants for host specificity testing and continued collecting pre-release data, including plant cover, density and height, at US field sites in anticipation of new agent releases.

Knapweeds: CPHST Albany continued work on optimization of a rearing system for *Cyphocleonus achetes* a root feeding weevil used for biological control of knapweeds. Detailed work on induction of reproductive diapauses was performed with the objective of developing an adult storing system. Initial results are indicating that storing adults for 2-3 months by manipulating light cycle may be possible without significantly affecting their reproductive potential.

Perennial Pepperweed & Canada Thistle: CPHST FCL initiated efforts in 2009 to survey for and assess the efficacy of plant pathogens attacking perennial pepperweed and Canada thistle in collaboration with CABI Europe-Switzerland and the University of California, respectively. In 2010, host specificity testing of a new race of white rust *Albugo candida* attacking perennial pepperweed was begun from collections made in CO and CA. Both CO and CA isolates showed a similar narrow host range that differed slightly. The Colorado isolate infected four hosts in addition to perennial pepperweed including *Iberis umbellata*, *Lepidum ruderale*, *Lepidum sativum*, and *Stanleya pinnata.* The California isolate infected these same four hosts and *Lepidium campestre*, *Lepidium draba*, and *Thelypodium integrifolium.* In 2010, a survey for fungal pathogens on Canada thistle was conducted in northwestern China. All material is currently being identified and assessed for its biocontrol potential. Thus far, a number of leaf-attacking fungal pathogens have been identified: powdery mildew, *Septoria* spp., *Phoma* spp., *Alternaria* spp., and *Puccinia* cf. *punctiformis*. The most promising pathogen collected to date is a white rust pathogen, *Albugo tragopogonis.* Varieties and physiological races of this fungus have been reported in the literature. Field observations suggest that it could be highly specific and damaging to *C.*

arvense. Inoculation tests on different *Cirsium* origins (China, USA) have been started and the U.S. biotypes of *C. arvense* have shown high susceptibility.

Russian Knapweed: A new Russian knapweed biological control agent, the gall midge *Jaapiella ivannikovi*, was permitted for US release in 2009. CPHST Mission Laboratory provided quarantine processing and rearing of *Jaapiella* galls received from Turkey and Uzbekistan. Initial releases in 2009 were made in WY and MT, and in 2010 CPHST FCL and local cooperators monitored these sites, confirming successful overwintering and establishment. A *Jaapiella* colony was initiated at FCL in 2010 using galls from Mission and Montana State University, and provided insects for several new releases in CO, in cooperation with Colorado Department of Agriculture. The FCL colony will be expanded in 2010-2011 to provide *Jaapiella* for new releases in additional western states in 2011.

Tropical Soda Apple: CPHST Miami worked with ARS Tallahassee, Florida Division of Plant Industry, and University of Florida (Ft. Pierce, Gainesville, Homestead) cooperators to evaluate the status of the leaf feeding beetle *Gratiana boliviana* released in FL to control the invasive pasture weed tropical soda apple (*Solanum viarum*). The group determined that the beetles were very effective in south and central Florida through studies of the beetle's impact as well as a survey conducted by Florida Agriculture and Mechanical University to determine stakeholder satisfaction with the program. By evaluating the 2009 release sites, the group also determined that the biological control agent did not establish well and exert the same control in north Florida. The group will conduct controlled field studies in 2011 at three different latitudes to determine what factors are limiting the beetles' success in the north.

Tropical Soda Apple: CPHST Miami worked with University of Florida scientists to test the host range of the leaf feeding beetle *Gratiana graminae*, a potential new biological control agent of tropical soda apple. In quarantine studies, the group found that the beetle fed and developed on Thai variety egg plant (*Solanum melangona*) and wild turkey berry (*Solanum torvum*) in addition to TSA. This cooperative effort generated information to help the Technical Advisory Group for Biological Control Agents of Weeds determine the risk of releasing this biological control agent in the field.

Yellow Toadflax: In 2009, CPHST FCL initiated a Colorado-based rearing effort with a yellow toadflax-adapted strain of the stem-mining weevil *Mecinus janthinus* in cooperation with Colorado State University (greenhouse-based rearing) and Colorado Department of Agriculture (field cage rearing). CSU completed development of rearing protocols in 2010 and transferred this technology to FCL, which scaled-up production and facilitated new releases of the weevil in ND, SD and OR. This project will provide *M. janthinus* for additional field releases in a number of other Western and Eastern Region states in 2011 and 2012.

INSECTS:

Asian Citrus Psyllid: CPHST Mission developed protocols for mass-production of *Tamarixia radiata* that are currently being tested in a USDA citrus grove. One mature citrus test tree has been shown to support the production of 21,600 parasitoids using a field insectary cage approach. In addition, newly collected colonies of *T. radiata* from Guangxi, Guangdong, Guangzhou, and Hong Kong were imported and established in the CPHST Mission Arthropod Quarantine Facility. A Pakistan strain of *T. radiata* is undergoing host-specificity testing at the Mission Laboratory alongside the work being done by the University of California-Riverside to meet the requirements of the permitting process. CHPST Mission also co-hosted the "USDA, APHIS International Workshop on *Tamarixia* Species" held in McAllen, TX, which included over 25 presentations and 60 attendees from all over the world. The workshop featured a tour of the Asian citrus psyllid biological control project at the Mission Lab.

Coffee Mealybug: CPHST Miami, in cooperation with APHIS-IS Santo Domingo, DR, the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF) and the Florida Agricultural and Mechanical University (FAMU), initiated a project to determine the impact of the coffee mealybug *Planococcus lilacinus* on local agricultural in the DR and whether natural enemies are providing any control for the pest. *P. lilacinus* has a broad host range and poses a significant threat to US ornamental and agricultural crops.

***Crypticerya genistae* Scale:** CPHST Mission continued efforts with PPQ Puerto Rico and the Puerto Rico Department of Agriculture in developing biological control options for a new invasive scale insect, *Crypticerya genistae*. A small adventive ladybird beetle (*Anovia circumclusa*) has been identified in both Barbados and Florida and is being evaluated as a potential biocontrol agent. Presence of the ladybird beetle was likewise confirmed in PR through field survey efforts. In addition, a predaceous phorid fly (*Syneura cocciphila* Coquillett) that is known to commonly feed on cottony cushion scale was recovered from *C. genistae*.

Emerald Ash Borer: CPHST Otis has been cooperating with scientists at USDA FS and ARS to develop biological control options for the ash borer (EAB). In 2010, CPHST Otis discovered new natural enemies of EAB in South Korea and the Russian Far East, including possibly new species of *Spathius, Tetrastichus,* and *Atanycolus*. These species are from colder climates and might provide superior control in the more northern parts of the U.S. and Canada. Rearing studies and host range testing of the new parasitoids were initiated and are on-going. Life-table studies of the factors (tree resistance, parasitoids) that control EAB in its native range (China, Russia) have begun. CPHST also continues to evaluate the establishment, spread and impact of existing BC agents being released. *Oobius, Spathius*, and *Tetrastichus* successfully overwintered at sites in MI, OH and MD. Research release sites have now been established in five states: IL (4), IN (1), MD (1), MI (11), and OH (2). Studies to develop methods to enhance the probability of parasitoid establishment (such as providing food for adults and girdling trees) have been initiated.

Grasshoppers & Mormon Crickets: CPHST Phoenix conducted research in a continuing joint effort with ARS and Utah State University on several domestic strains of fungal pathogens, *Metarhizium anisopliae* and *Beauveria bassiana*, for control of grasshoppers and Mormon crickets. The *Metarhizium* strains, F52, DWR-356 and DWR-346 and the *Beauveria* strain GHA were tested against Mormon cricket in Sidney, MT, with ARS using the Field Aerial Application Spray Simulation Technique on 576 mini plots. All of the *Metarhizium* strains were tested against grasshoppers near Howell, UT, with Utah State University on four 10-acre plots, using ULV ground application technology recently developed by CPHST Phoenix. Both studies were in support of development and registration of a domestic strain of *Metarhizium* suitable for deployment against domestic rangeland pests and were in preparation for future large scale field trials anticipated in 2011.

Grasshoppers & Mormon Crickets: CPHST Phoenix applied for permits to release and evaluate the exotic biopesticides Green Guard from Australia and Green Muscle from Africa for control of grasshoppers and Mormon crickets in the US. The products are based on different strains the entomopathogenic fungus *Metarhizium anisopliae.* The Environmental Assessment (EA) is currently open and available for comment. The permits will allow 10-acre applications. Depending on public comments to the EA, the current plan is to conduct field evaluations in 2011 in one of five western states,with the primary location being either Custer or Prairie Co., MT. Techniques for both aerial and ground application have been tested and are available for the trials.

Harrisia Cactus Mealybug: CPHST Mission continued efforts with PPQ Puerto Rico, Puerto Rico Department of Agriculture, University of Puerto Rico, and the Barbados Ministry of Agriculture to identify natural enemies to combat harrisia cactus mealybug *Hypogeococcus pungens*, a significant threat to native and endangered columnar cacti in PR. Studies of the biology and genetics of a new species of parasitoid wasp *Leptomastidea* nr *antillicola* Dozier (Hymenoptera: Encyrtidae) are ongoing. Information gathered in these studies will determine whether or not the parasitoid should be considered for mass rearing and release efforts.

Harrisa Cactus Mealybug: CPHST Miami organized and conducted surveys for natural enemies of the cactus mealybug *H. pungens* from areas in FL where the pest was previously collected. A total of 58 locations in 17 counties were surveyed. Out of 58 locations, only 9 locations had *H. pungens* infestations, 6 of which contained parasitized *H. pungens.* The study indicated that the mealybug is under good biological control and the identified predators and parasitoids may work as classical biological control agents against the cactus mealybug in PR.

Imported Fire Ant: CPHST Gulfport continued to coordinate the APHIS-funded IFA Phorid Fly (*Pseudacteon* spp.) rearing and release program. In 2010, multiple releases of a third fly species, *P. obtusus* and production rearing of a fourth species *P. cultellatus* were initiated. A publication on the establishment and spread success of the first 2 species, *P. tricuspis* and *P. curvatus* was accepted for publication by J. Insect Science. Data shows that both species are established in more than 50% of the IFA quarantined area.

SUMMARY OF ACCOMPLISHENTS (CONTINUED)

Light Brown Apple Moth: CPHST Moss Landing initiated a project together with the California Department of Food and Agriculture to assess the ability of *Trichogramma platneri* to suppress light brown apple moth (LBAM) populations alone and combined with sterile moth releases. Percent eclosion of LBAM eggs in control field cages containing only fertile moths was 92%, compared with 16% when sterile moths were added at a 10:1 sterile to fertile overflooding ratio and 2% when both sterile moths and parasitoids were added.

Light Brown Apple Moth: CPHST Albany continued work on biological control of LBAM in cooperation with CDFA and UC Berkeley scientists. Rearing systems for two species of *Trichogramma* (*T. platerni and T. fasciatum*) were established from which commercial colonies may be developed. Systematic field observations of the seasonality of these two natural enemies were carried out using UV irradiated LBAM eggs as trap hosts for screening.

Mediterranean Fruit Fly: CPHST Guatemala continued to conduct field tests to evaluate and optimize combined releases of the egg-parasitoid *Fopius ceratitivorous* and sterile Mediterranean fruit flies. In 2010 ground releases of this parasitoid together with aerial releases of sterile male only medflies were conducted under open field conditions in an effort to establish a recommended release density of the parasitoid. Results from these experiments are currently being analyzed.

Red Palm Weevil: CPHST Miami continued to work on a variety of offshore safeguarding initiatives. They determined population densities, distribution, survey methodologies, control options and impact of a recently introduced pest of palms, the red palm weevil (*Rhynchophorus ferriguneus*), in Aruba and Curacao. Results of these studies were communicate to PPQ-CPHST, APHIS IS, Greater Caribbean Safeguarding Initiative and PPQ EDP, and incorporated into the New Pest Response Guide Lines for this pest. These recommendations are now being used to combat the RPW infestation recently detected in southern CA.

Sirex Woodwasp: CPHST Otis continued to mass rear Sirex nematodes and carried out experimental releases of the Australian nematode strain against *Sirex noctilio* at field sites in NY and PA. This season's work compared infection rates of the Australian and "native" strains in three pine species and investigated the effect of release date on nematode infection rate. Laboratory studies investigated the possible hybridization of the two nematode strains, molecular techniques for discriminating the strains, and the effects of fungus strain, initial nematode numbers, and length of rearing on final nematode yields.

Biological Control of Canada Thistle

LOCATION: Fort Collins Lab
CPHST STAFF: Rich Hansen (Lead); Christina Southwick (Support)
CHAMPIONS: Shaharra Usnick (WR Program Manager); Ron Weeks (ER Program Manager)
CONTACT: Rich Hansen (richard.w.hansen@aphis.usda.gov)

Canada thistle, *Cirsium arvense* (Asteraceae), is among the most economically and environmentally damaging exotic weeds in the US. Biological control remains a potentially useful management tool that has achieved only sporadic success against Canada thistle infestations. The search for new, effective agents continues. 2010 activities are summarized with a native lace bug and an accidentally-introduced rust mite that feed on Canada thistle in the US, assessing their utility in a biocontrol program.

Assessment of a native lace bug as a potential Canada thistle biocontrol agent. The distinct lace bug, *Corythucha distincta* (Hemiptera: Tingidae) (**Fig. 1**) is a native insect that feeds on Canada thistle, occasionally causing leaf and shoot mortality. We have been examining the possibility of employing this insect as a biological control agent for the weed, beginning in 2008. The first objective in this project is an assessment of the host specificity of *C. distincta*, comparing lace bug feeding and survival on Canada thistle with that on native or crop plants. No-choice and host-choice laboratory and host-choice field cage experiments conducted in 2009 showed that lace bug utilization of native *Cirsium* thistles was similar to, and often exceeded that, of Canada thistle (CPHST FCL 2009 Annual Report). In addition, crop plants related to thistles (artichoke, cardoon, safflower, and sunflower) were not utilized by *C. distincta*.

Figure 1. Distinct lace bug, *Corythucha distincta*, adults and nymphs.

In 2010, we continued laboratory host-choice tests with Canada thistle, 7 native *Cirsium* thistles (including one, *C. neomexicanum*, tested for the first time), and two related exotic thistles (Scotch thistle, *Onopordum acanthium*, and cardoon, *Cynara cardunculus*). Tests used methods previously described (CPHST FCL 2009 Annual Report) and we used *C. distincta* adults from an FCL lab colony or collected from nearby field populations. Lace bugs occurring on various test plants were counted at 7 and 14 days after introduction.

Four replicates were completed in 2010. As in 2009, utilization of most native *Cirsium* spp. was equivalent to, or exceeded, that of Canada thistle (**Fig. 2**). *O. acanthium* was utilized at a very low level, and *C. cardunculus* was not attacked. When combining 2009 and 2010 host utilization data, lace bugs were more often found on three native thistles (*C. centaureae*, *C. occidentale*, and *C. tracyi*) than on Canada thistle, and found as frequently on the other five tested native thistles as on Canada thistle (**Fig. 3**). Use of the 'other' exotic true thistles (*Silybum*, *Onopordum*, and *Cynara*) was similar to, or less than, that of *C. arvense*. Related crop plants outside of the subtribe Carduinae (safflower, *Carthamus tinctoria*, and sunflower, *Helianthus annuus*) were not utilized by lace bugs.

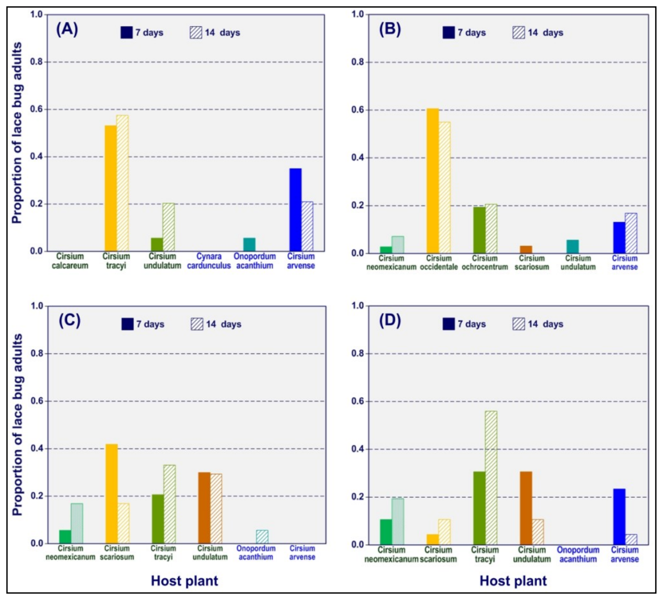

Figure 2. Relative occurrence of *Corythucha distincta* adults on plants in 2010 lab host-choice experiment, four replicates (n = 35 adults for A; n = 50 adults for B, C, and D).

We also conducted a field colonization experiment in August and September 2010. Nine test plants, including Canada thistle, five native *Cirsium* thistles, and Scotch thistle, where randomly planted in a Canada thistle infestation in Fort Collins, CO that supported a 'moderate' population of *C. distincta*. Once a week for 8 weeks, lace bug nymphs and adults were counted and feeding damage scored on each test plant; feeding was estimated on a 0-3 scale (0 = no feeding, 1 = light feeding, 2 = moderate feeding, and 3 = significant feeding and foliar discoloration). Unfortunately,

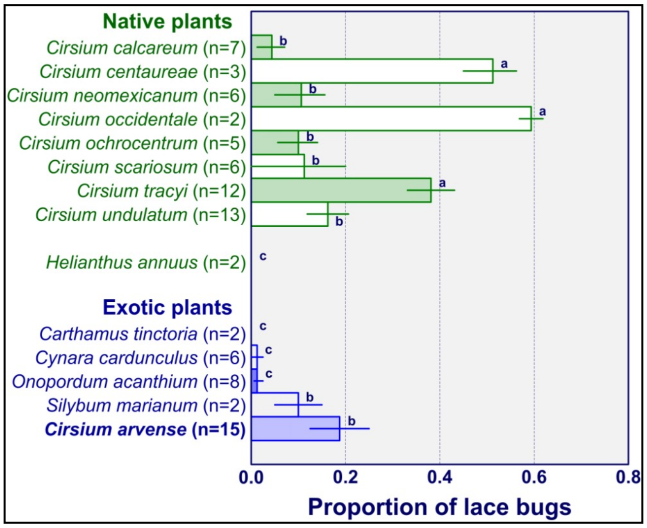

Figure 3. Mean occurrence (± SE) of *C. distincta* adults on test plants, 2009 / 2010 lab host-choice experiments. means followed by the same letters are not significantly different.

Canada thistle plants were heavily defoliated by grasshoppers and quickly died. All five native *Cirsium* spp. supported nymphal and adult lace bug populations and some level of observable feeding damage throughout the experiment (**Table 1**); feeding was most significant on *C. tracyi*. A few lace bug adults (but no nymphs) were found on Scotch thistle, and no feeding damage was observed.

Earlier reports listed Canada thistle and other exotic *Cirsium* and *Carduus* thistles (not tested here), two native *Cirsium* thistles, as well as plants from Fabaceae, Malvaceae, and several other plant families as *C. distincta* hosts. In general, many lace bugs are believed to have comparatively narrow host ranges. Our experiments show that native *Cirsium* thistles are probably the ancestral hosts of this native insect, with exotic *Cirsium* thistles also utilized; other exotic true thistles (e.g. *Carduus*, *Onopordum*, *Silybum*) may be marginal hosts. More distantly related plants in Asteraceae (e.g. sunflower and safflower) are not utilized by *C. distincta*, and host records from other plant families appear erroneous. Feeding on native *Cirsium* thistles probably precludes applied utilization of *C. distincta* as a biocontrol agent. However, the final objective of this study is a quantitative assessment of *C. distincta* impacts on Canada thistle and nontarget plants; anecdotal observations indicate that lace bug feeding can stunt or kill Canada thistle shoots when populations are high. Work will be completed in 2011.

Utilization of native *Cirsium* thistles by the Canada thistle rust mite. Efforts to document nontarget utilization of native thistles and related plants by *Aceria anthocoptes* continued in 2010, when we sampled nine Asteraceae plants for mites. These included four native *Cirsium* thistles, a native sunflower, and four exotic species (including Canada thistle). Among the native thistles, one was sampled for the first time: Wyoming thistle, *Cirsium pulcherrimum* (collected in Albany and Fremont Counties, Wyoming). Putative *A. anthocoptes* mites were collected from three of four native *Cirsium* spp. (**Table 2**).

2010 sampling confirmed that no mites have been collected from any plants outside the genus *Cirsium*. Mite samples from *C. canescens*, *C. eatonii*, and *C. pulcherrimum* are awaiting taxonomic confirmation by Dr. Radmila Petanovic, University of Belgrade (Serbia).

Table 1. Lace bug colonization and feeding damage during 2010 field experiment.

	Native species																		Exotic species								
Week	*Cirsium neomexicanum*			*Cirsium occidentale* 1			*Cirsium occidentale* 2			*Cirsium ochrocentrum*			*Cirsium tracyi*			*Cirsium undulatum*			*Cirsium arvense* 1			*Cirsium arvense* 2			*Onopordum acanthium*		
	Ad	Ny	FS	Ad	Ny	FS	Ad	Ny	FS	Ad	Ny	FS	Ad	Ny	FS	Ad	Ny	FS	Ad	Ny	FS	Ad	Ny	FS	Ad	Ny	FS
1				1	4	0							6	60	2	11	17	1	0	0	0				0	0	0
2				3	3	0							41	200	2	17	13	1				1	0	0	0	0	0
3	6	1	0	9	0	0	10	41	1	3	6	0	31	110	2	43	11	1				1	0	0	0	0	0
4	15	1	0	5	0	0	22	41	2	15	7	1	81	59	3	42	10	1							0	0	0
5	9	1	0	8	3	0	43	4	2	21	1	1	101	52	3	66	0	1							2	0	0
6	30	0	1	18	0	1	57	0	2	44	1	2	112	32	3	64	0	2							8	0	0
7				4	0	1	46	2	2	38	0	2	86	17	3	85	0	2							2	0	0
8				2	0	1	38	0	3	42	0	2	33	1	3	93	0	2							11	0	0

Ad = number of lace bug adults **Ny** = number of lace bug nymphs **FS** = feeding score (0 = none; 1 = light; 2 = moderate; 3 = significant)

Table 2. Collection and identification of *Aceria anthocoptes* from various hosts in Colorado and Wyoming.

	Aceria mites coll'd (year)?					
Host plant	**2006**	**2007**	**2008**	**2009**	**2010**	**Positive ID?**
Native thistles						
Cirsium barnebyi			+	+		Yes
C. calcareum		+	+	+		Yes
C. canescens				+		?
C. centaureae	+	+	+	+		Yes
C. eatonii				+	+	?
C. neomexicanum				–		
C. ochrocentrum		+	+	+	–	Yes
C. scariosum	+	+	+	+	+	Yes
C. pulcherrimum					+	?
C. scopulorum	+	+	+	+		Yes
C. tracyi		–	+	+		Yes
C. undulatum	+	+	+	+		Yes
Exotic thistles						
Cirsium arvense	+	+	+	+	+	Yes
C. vulgare	–	–				
Carduus nutans	–	–		–		
Onopordum acanthium			–	–	–	
Other Asteraceae						
Acroptilon repens (exotic)					–	
Helianthus annuus (native)					–	
Lactuca serriola (exotic)					–	

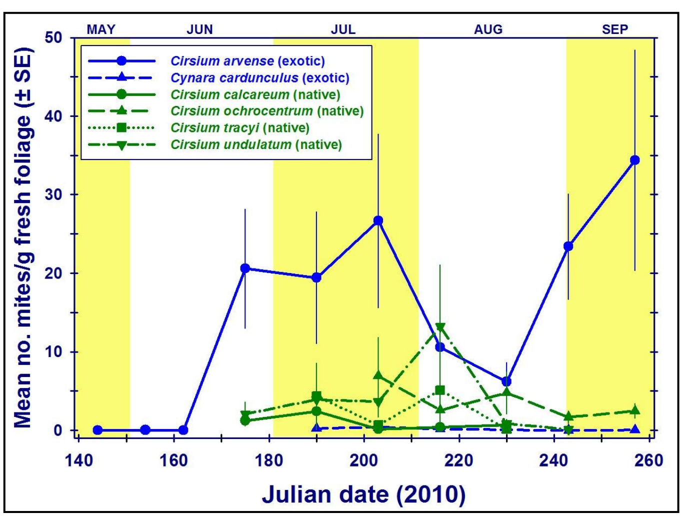

Figure 4. Abundance of *Aceria anthocoptes* on native and exotic thistles in 2010 (USDA-ARS farm, Ft. Collins, CO).

Our thistle garden, located at the USDA-ARS farm northeast of Fort Collins, was sampled for *A. anthocoptes* abundance biweekly from late May through mid-September 2010. This experiment was established to assess among-host differences in relative mite abundance and seasonal population patterns under field conditions. Methods used were generally the same as those employed in 2009 (CPHST FCL 2009 Annual Report). In 2010, we collected five samples per plant for each host, and only six species had enough garden replicates to support this sampling intensity: four native *Cirsium* thistles, Canada thistle, and the cultivated thistle *Cynara cardunculus* (cardoon). Mite counts were significantly higher on Canada thistle than on any native thistle for every sampling date except August 4 and 18 (**Fig. 4**). No mites were extracted from cardoon.

Results from 2010 confirm earlier observations that *A. anthocoptes* only utilizes thistles in the genus *Cirsium*, and at least 12 native *Cirsium* thistles are mite hosts. In 2011 we will continue to sample additional native thistles for the presence of *A. anthocoptes*.

Assessing nontarget plant utilization by the Canada thistle stem gall fly, *Urophora cardui*. In early 2010, we received a question about possible utilization of several native *Cirsium* thistles by the stem gall fly, *Urophora cardui* (Diptera: Tephritidae). This fly is a permitted Canada thistle biocontrol agent that was first released in 1980. *U. cardui* has been widely released in the US and scattered populations are present in many eastern and western states. Larval feeding induces the formation of a stem gall that stunts shoot growth and reduces flower and seed production, but typically does not kill Canada thistle stems or plants (**Fig. 5**). *U. cardui* is believed to be host-specific and nontarget attacks have not been reported in the literature. However, pre-release host specificity tests included only a few native *Cirsium* thistles (more than 90 occur in the US), and, to our

Figure 5. *Urophora cardui* stem gall on Canada thistle, Ft. Collins, CO, July 2010.

knowledge, no formal post-release lab or field studies of *U. cardui* galling of native plants have been conducted.

We conducted a preliminary study to assess possible non-target galling. In July 2010, Canada thistle (n=6) and two native thistles, *Cirsium ochrocentrum* (n=5) and *C. occidentale* var. *venustulum* (n=2), plants were randomly planted in 2 x 2 x 2 m field cage (**Fig. 6**). Both native thistles are sympatric with Canada thistle populations in the western US. Overwintering *U. cardui* galls were collected in western Colorado in early spring 2010 and held in cold storage until July, when they were placed in a growth chamber at 25°C and a 16:8 hrs light:dark photoperiod until adults eclosed. A total of 59 adult flies were then released in the cage on July 26, 2010; more than half of the flies were females. Plants were watered at least once a week until September 14, 2010 (50 days), when thistle plants were harvested and examined for galls. Three of the six Canada thistles had a single stem gall, while no galls occurred on the native *C. ochrocentrum* and *C. occidentale*. Thus, this simple study provides additional evidence that *U. cardui* does not attack native thistles, at least in a host-choice environment. We hope to repeat this experiment in a more carefully replicated manner and with additional native thistles in 2011.

Figure 6. *Urophora cardui* host choice experiment field cage, ARS farm (July 2010).

Survey for Natural Enemies of Canada Thistle (*Cirsium arvense*)

LOCATION: Fort Collins Lab
CPHST STAFF: Melinda Sullivan (Lead) (Fort Collins) and Christina Southwick (Support)
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Canada thistle (*Cirsium arvense*), exotic to North America, Australia, New Zealand and South Africa, is an aggressive perennial weed that competes with other plants for water, nutrients, and space and reduces the grazing capacity and diversity of rangeland and natural areas. *C. arvense* is a listed noxious weed in at least 28 states and is the most frequently-listed weed in the United States. A variety of herbicides are currently labeled for use against *C. arvense* but are not effective for non-agricultural use due to logistical concerns, environmental concerns, and the perennial growth habit of Canada thistle.

Biological control is currently being investigated as part of an integrated pest management program for Canada thistle. The search for natural enemies of Canada thistle has focused on Europe, the reported center of origin of *C. arvense*. However, evidence suggests that the range of *C. arvense* may be broader than originally thought, reaching into North Africa and western Asia. Indeed, recent surveys undertaken in China have revealed the occurrence of a wealth of natural enemies (both arthropods and pathogens), some as yet to be considered for classical biological control. *C. arvense* is considered to be at most an occasional problem weed in China and northern India. This indicates that these eastern regions may be an important center of diversity of the species, and that it could prove valuable to investigate the potential of new agents that occur there. Several arthropods have been introduced into the United States but their success on Canada thistle has been extremely variable from year to year and largely unsuccessful. No plant pathogen agents have been deliberately introduced, although a number of pathogens have been recorded from its native and introduced range.

In 2009, CPHST Ft. Collins initiated efforts to survey for and assess the efficacy of plant pathogens attacking Canada thistle in collaboration with CABI Europe-Switzerland. CABI and its partners proposed pathogen surveys in the Xinjiang Autonomous Region of northwestern China in the summer of 2009; this region supports a diverse *Cirsium* flora and is climatically similar to much of the continental United States. Political unrest in Xinjiang, however, precluded access. Instead, 22 sites in western Mongolia were surveyed. A variety of pathogens were collected, but it appears that the Mongolian hosts were the closely-related *C. setosum* rather than *C. arvense*. Leaf samples were collected to confirm this using molecular analyses.

In 2010, a survey for fungal pathogens on *C. arvense* was conducted in northwestern China. All material is currently being identified and assessed for its biocontrol potential. Thus far, a number of leaf-attacking fungal pathogens have been identified: powdery mildew, *Septoria* spp., *Phoma* spp., *Alternaria* spp., and *Puccinia* cf. *punctiformis*. Despite reports that up to four rust species occur on *C. arvense* in Xinjiang Province, initial findings suggest that only one species, the systemic rust *Puccinia* cf. *punctiformis,* is actually present. The other rust species may be synonyms or on closely related but misidentified thistle hosts. Since this species is already present in North America (accidentally introduced), its potential as a classical biological agent is limited unless more aggressive strains can be identified.

A promising pathogen is a white rust, *Albugo tragopogonis* (**Fig. 1**). Although this pathogen has been reported as occurring in the United States on sunflower, different varieties and physiological races of this fungus have been reported in the literature. Further studies are needed to ascertain if the Chinese pathogen is a different variety or race than that occurring in the United States. Field observations suggest that it could be highly specific and damaging to *C. arvense*. Inoculation tests on different *Cirsium* origins (China, USA) have been started and the U.S. biotypes of *C. arvense* have shown high susceptibility.

Figure 1. White rust pustules of *A. tragopogonis* on Canada Thistle. Photos courtesy of CABI.

Pre-Release Research and Development Efforts for PPQ Weed Biocontrol Targets: Project Updates

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Introduction. Before classical biological control can be implemented against an exotic weed target, potential biological control agents must be identified, collected, studied for efficacy, and screened for host specificity (i.e. their risk to nontarget native US and crop plants assessed). This report summarizes the current status of research and development efforts addressing 10 weed targets prioritized by PPQ. All were identified during PPQ target canvassing efforts in 1997 (Russian knapweed and Canada thistle), 2000 (field bindweed, hoary cress, and garlic mustard) and 2005 (houndstongue, dyer's woad, perennial pepperweed, yellow toadflax, and hawkweeds). This work has been conducted by CABI Bioscience (Delémont, Switzerland), along with other European and Asian cooperators. USDA-ARS is collaborating in pre-release research addressing hoary cress, Russian knapweed, and hawkweeds. CPHST Fort Collins funded pre-release research the biocontrol projects described below in 2010.

Dyer's wood, *Isatis tinctoria* (Brassicaceae). Biocontrol research and development efforts targeting this weed began in 2005, with natural enemies surveyed in central Europe, Turkey, Russia, and Kazakhstan. CABI continued pre-release research with the three most promising prospective biocontrol agents in 2010. Two of these agents show the ability to kill dyer's woad plants: the root crown-mining weevil *Ceutorhynchus rusticus* (**Fig. 1**) and the stem-mining flea beetle *Psylliodes isatidis. C. rusticus* shows an apparently high degree of host specificity; in tests through 2010, it has been able to complete at least limited development on only 5 of 78 nontarget host plants tested, more than half of which were North American natives. *P. isatidis* appears somewhat less host-specific, as tests through 2010 indicate that 33 of 70 nontarget plants could support some *P. isatidis* larval development in no-choice tests; nontarget utilization is much lower in choice tests. However, field tests documenting flea beetle attack on nontarget test plants may actually involve contamination by other flea beetles. The third agent in development is the seed-feeding weevil *C. peyerimhoffi*. Host specificity testing with this insect occurred in 2009 and 2010, and preliminary results indicate that it is very host-specific. Host specificity experiments with all three agents will continue in 2011, with possible completion of research with *C. rusticus* and *P. isatidis*.

Figure 1. *Ceutorhynchus rusticus* larvae in dyer's woad root crown (CABI Europe).

Garlic mustard, *Alliaria petiolata* (Brassicaceae). The search for potential garlic mustard biocontrol agents started in 1998. Host specificity tests with the most promising potential agent, the root-mining weevil *Ceutorhynchus scrobicollis*, were completed in 2008. These experiments showed that the weevil developed on only 5 of 79 plants studied in no-choice or host choice environments, and no native US plants tested were utilized. A release petition was submitted to the weed biocontrol Technical Advisory Group (TAG) in 2008, but TAG recommended in early 2009 that *C. scrobicollis* not be released in the US, primarily due to concerns about possible utilization of several native mustards that were not tested and some utilization of watercress (*Nasturtium officinale)*. CABI conducted additional host specificity experiments in 2009 and 2010; hopefully, these will be completed in 2011, when the release petition could be resubmitted.

Other potential garlic mustard agents in development include three additional *Ceutorhynchus* weevils, and pre-release research continued in 2010. Host-specificity experiments with two shoot-mining weevils, *C. alliariae* and *C. roberti*, will, hopefully, be completed in 2011 with possible submission of US release petitions in 2012; both agents seem to have very narrow host ranges. A seed-feeding weevil, *C. constrictus*, may be a useful biocontrol agent since garlic mustard is a biennial plant reproducing only by seeds. *C. constrictus* appears quite host-specific, and pre-release testing should be completed with 2011 experiments; a petition could be submitted in late 2011 or 2012.

Hoary cress, *Lepidium draba* (Brassicaceae). CABI is conducting pre-release research on four possible hoary cress biocontrol agents. The agent closest to completion is the stem-galling weevil *Ceutorhynchus cardariae* (**Fig. 2**). In host-specificity tests conducted from 2003 to 2010, this insect was able to complete development on 12 (including 7 US natives) of 93 nontarget plants tested in no-choice experiments but developed only on a single US native plant in lab or field choice tests. This plant is San Diego pepperweed, *Lepidium latipes*, an annual that will not sustain *C. cardariae* populations in the field. No tested crop plants were suitable weevil hosts. Host specificity tests will be completed, and a release petition submitted to TAG in 2011. The seed-feeding weevil *C. turbatus*, appears to be the most host-specific prospective hoary cress agent. In experiments conducted from 2003 to 2010, this insect did not complete development on any native US or crop plant tested. Host-specificity tests with *C. turbatus* should also be completed in 2011, with possible submission of a TAG petition in 2011. *C. turbatus* can reduce hoary cress seed production and spread but will not kill plants.

Figure 2. Hoary cress stem-galling weevil, *Ceutorhynchus cardariae*, adult (CABI Europe).

The two remaining potential hoary cress agents are the root mining weevil *Melanobaris* sp. near *semistriata* and the shoot-mining flea beetle *Psylliodes wrasei*. Host-specificity experiments concluded in 2010 showed that both attacked a number of native US test plants. Thus, these insects are considered to have unacceptably broad host ranges, and will no longer be considered for US release. In 2011, additional natural enemy surveys for new potential hoary cress agents may be conducted in eastern Russia, Turkey, and/or Uzbekistan. CABI is also considering renewing pre-release research with another stem-mining weevil, *C. merkli*.

Perennial pepperweed, *Lepidium latifolium* (Brassicaceae). In 2006 and 2007, CABI conducted natural enemy surveys in Turkey, Russia, Kazakhstan, China, and Russia to identify potential perennial pepperweed agents. Host specificity tests with three prospective insects (begun in 2008) continued in 2010. The stem-galling weevil *Ceutorhynchus marginellus* was collected in Russia, China, and Kazakhstan. Successful development occurred on 5 of 32 nontarget plants in no-choice tests, including three native US plants. In lab and field choice experiments, galling occurred on three native plants (alkali pepperweed, *L. crenatum*; common pepperweed, *L. densiflorum*; Huber's pepperweed, *L. huberi*) but these plants were attacked much less frequently than the target weed. Host-specificity testing will continue in 2011, and possible impacts of *C. marginellus* on *L. crenatum* and *L. huberi* will also be studied.

The stem-mining flea beetle *Phyllotreta reitteri* was collected in Kazakhstan and Russia, where it kills perennial pepperweed stems. No-choice experiments conducted from 2007 through 2010 show that *P. reitteri* completed development on 15 of 39 plants tested to date, including limited development on eight native US species. However, nontarget plants were not attacked in lab or field host-choice tests. Host-specificity tests continue in 2011.

Using molecular analysis, the root-mining weevil *Melanobaris* sp. near *semistriata* is distinct from the *Melanobaris* weevil on hoary cress (see previous section). Host specificity experiments conducted from 2007 through 2010 show that this insect was unable to complete development on any of the more than 40 nontarget plants, including more than 20 native US species (no-choice and host-choice tests). However, oviposition and limited larval feeding was observed on some nontargets. Field host-choice experiments will continue in 2011 and a decision about whether this insect is host-specific enough for US release will be made.

In 2011, host specificity experiments will be initiated for two additional arthropods from Turkey: the gall mite *Metaculus lepidifolii* and the stem-mining fly *Lasiosina deviata*.

Field bindweed, *Convolvulus arvensis* (Convolvulaceae). In 2009, CABI and its eastern European partners initiated a new field bindweed natural enemy survey, primarily seeking potential root- and stem-feeding agents. The most promising are a shoot- and root-mining fly, *Melanagromyza albocilia*, and the root-mining flea beetles *Longitarsus pellucidus* and *L. rubiginosus*. Host specificity experiments with these insects will continue in 2011.

Houndstongue, *Cynoglossum officinale* (Boraginaceae). In 2010, pre-release research continued with the most promising houndstongue biocontrol agent, the seed-feeding weevil *Mogulones borraginis*. This weevil appears to be the most host-specific houndstongue agent, and is able to reduce seed production by up to 50%. In 2010, CABI completed feeding tests with the seed weevil and critical native plant *Cynoglossum grande* (Pacific houndstongue); no oviposition or feeding were observed on this native plant in host choice experiments. We anticipate submission of a US release petition in 2011.

Russian knapweed, *Acroptilon repens* (Asteraceae). Work on possible new agents continued in 2010. The bud gall mite *Aceria* sp. near *acroptilonica* appears to be a host-specific agent that affects shoot tips and flower buds, stunting shoots and reducing flower and seed production. In 2010, field host-choice tests were conducted in Iran with European and Asian nontarget plants; no galling occurred on any plants aside from *A. repens*. Host-specificity experiments using North American plants will be conducted in 2011, and ongoing molecular analyses will hopefully be completed to allow positive identification of this mite. Host-range testing with a leaf-feeding beetle, *Galeruca* sp., from Uzbekistan was initiated in 2010. Initial results suggest that possible nontarget hosts may include only *Centaurea* spp. and *Saussurea* spp. In 2011, experiments will expand to include North American plants, especially native *Centaurea*.

In 2010, CABI suspended pre-release research with root-boring moth *Cochylimorpha nomadana*, a potentially effective agent that is very difficult to work with in the lab or in the field. CABI is also considering a new Russian knapweed natural enemy survey in western Iran in 2011.

Figure 3. Stem galls on yellow toadflax induced by *Rhinusa pilosa* (CABI Europe).

Yellow toadflax, *Linaria vulgaris* (Scrophulariaceae). CABI continued pre-release research with two promising potential biocontrol agents in 2010: the stem-galling weevil *Rhinusa pilosa* (**Fig. 3**) and the stem-mining weevil *Mecinus heydeni*. Both agents appear to have significant negative impacts on yellow toadflax, including stem and plant mortality, and are quite host-specific. No-choice host specificity experiments with *R. pilosa* have employed 75 nontarget plants to date, including 37 native US species. Oviposition and limited galling was observed on four native plants, with complete development rarely observed on only one native, *Sairocarpus virga*. No development occurred on native plants in host-choice tests. In 2011, host-specificity testing will finish and a petition for US field release will be submitted. *M. heydeni* has been tested on 37 plants, including 21 native species. Complete development has been observed on two native plants, *S. virga* and *Nuttallanthus canadensis* in no-choice experiments; oviposition was recorded on *N. canadensis* in host-choice tests. *M. heydeni* host specificity experiments will continue in 2011 and may be completed by 2012. Because of recent taxonomic revisions of the Scrophulariaceae and related families, a revised host plant test list for prospective yellow and Dalmatian toadflax biocontrols should be completed in 2011.

Hawkweeds, *Pilosella* spp. (Asteraceae). Orange hawkweed (*Pilosella aurantiaca*) and meadow hawkweed (*P. caespitosa*) are the two primary exotic hawkweeds in the US that are targets for biocontrol agents. The bud gall wasp *Aulacidea subterminalis* was approved for US field release in late 2010, the first permitted hawkweed biocontrol agent; hopefully, US releases can begin in 2011. Host specificity testing continued with the stem-mining fly *Cheilosia psilophthalma* in 2010, and will continue in 2011. Host specificity experiments with the root-mining fly *C. urbana* continued in 2010. To date, 63 nontarget plants, including 36 native North American species have been tested; several native *Hieracium* hawkweeds supported limited development. A release petition may be submitted to TAG in 2011. However, pre-release research with a fourth prospective biocontrol agent, the stem gall wasp *Aulacidea hieracii*, was terminated after 2010 experiments confirmed utilization of native US hawkweeds (*Hieracium* sp.) and logistic difficulties in rearing and testing this insect.

Russian Knapweed and Yellow Toadflax Biocontrol Project Updates

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Biocontrol of Russian Knapweed (*Acroptilon repens*)

Field releases of the bud gall midge *Jaapiella ivannikovi*. The bud gall midge *J. ivannikovi* (Diptera: Cecidomyiidae) was permitted for US field release in 2009. Initial releases were made at several sites in Montana in early summer 2009 by Montana State U. (MSU) personnel. Additional releases were made near Riverton (Fremont Co., WY) and Powell (Park Co., WY) in May, June, and September 2009 (CPHST FCL 2009 Annual Report). In May and June 2010, we visited the Wyoming release sites. Galls were discovered at both Fremont Co. (**Fig. 1**) and Park Co. sites, documenting successful overwintering. Visual surveys found that most galls occurred near the 2009 release points, but some galls were found up to 30 m away, indicating at least limited adult *J. ivannikovi* dispersal. The 2010 site visits by Montana partners also confirmed successful *J. ivannikovi* overwintering in Montana. Galls were also released in a field cage at the Colorado Department of Agriculture (CDA) laboratory in Palisade (Mesa Co., CO) in 2009. However, post-release monitoring in 2009 and 2010 found no galls. Galls used in the 2009 releases originated from a lab colony at MSU and a quarantine colony at CPHST Mission Lab.

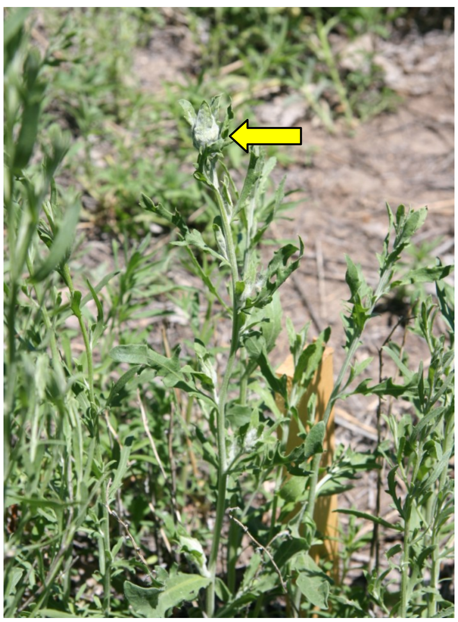

Figure 1. *Jaapiella ivannikovi* gall* at Riverton, WY 2009 release site, June 2010.

*The yellow arrow indicates the gall, and the yellow stake indicates post-release monitoring point.

In 2010, *J. ivannikovi* galls from MSU and a new lab colony at our CPHST location (see next page) were used to augment existing Wyoming releases and to initiate new releases in Colorado. For the latter, releases were made at the USDA-ARS farm near Fort Collins, Larimer Co., and at the CDA laboratory in Palisade, May–July 2010. At the ARS farm site, galls were released in four 1 m³ fine-mesh screen cages. In the initial cage (established on May 21), galls were evident within the cage and outside the cage by early June (**Fig. 2**); external galls were observed up to 20 m from the release cage. Post-release galling was not observed for other caged releases made in June and July 2010. The site received little or no rainfall during the summer, and Russian knapweed plants appeared stressed and senescent by mid-summer; this may have inhibited later gall formation. Post-release galling was evident at the CDA site during summer 2010. We will visit both Colorado locations in 2011 to assess overwintering and establishment.

Establishment of a *Jaapiella ivannikovi* lab colony at Fort Collins CPHST. In early spring 2010, we initiated a small-scale rearing program at FCL. Potted Russian knapweed plants originating from seeds or from field-collected rootstocks were grown under artificial lighting (16:8 h light:dark). Galls were obtained from MSU at various intervals and placed in fine-mesh screen cages with 5-10 bolting knapweed plants. Cages were reared in growth chambers or in the lab at ca. 25°C and 16:8 h photoperiod. Progeny galls were collected 3-4 weeks later and used for field release or to initiate new rearing cages. We reared two complete generations and a partial third in this manner in spring 2010, but productivity was very low, due primarily to the small stature and poor vigor of lab-grown hosts. About 30 galls were available for field release in May and June 2010. We will greatly expand the rearing in 2011. Galls will be provided for field release in spring and summer 2011.

Status of *Aulacidea acroptilonica*. The bud gall wasp *A. acroptilonica* (Hymenoptera: Cynipidae) was permitted for US release in 2008, the first biocontrol approved for Russian knapweed. Pre-release quarantine rearing is required with this insect; galls collected in its native Asian range are typically infested with a variety of parasitoids, predators, or inquilines, preventing their direct release. Difficulties in rearing this insect (CPHST Mission lab) have prevented any US field releases through 2009, and in 2010 we were unable to obtain viable *A. acroptilonica* galls from cooperators in Turkey and Uzbekistan. Hopefully, galls will be available in 2011, providing insects for propagation and field release.

Figure 2. *Jaapiella ivannikovi* release cages at ARS farm (left); interior of cage showing post-release galling, June 2010, with yellow arrows indicating galls (right).

Biocontrol of Yellow Toadflax (*Linaria vulgaris)*

Field releases of *Mecinus janthinus*. Established populations of a yellow toadflax-adapted stem-mining weevil *M. janthinus* (Coleoptera: Curculionidae) 'strain' were confirmed at several western Montana sites (CPHST FCL 2009 Annual Report). In late April 2010, adult weevils were collected from several Powell Co. locations by Montana PPQ, USDA Forest Service, and MSU project partners and distributed for field release in Colorado (four sites; see below), North Dakota (two sites), South Dakota (two sites), and Wyoming (two sites).

In Colorado, a caged 2009 *M. janthinus* release was made by CDA personnel in Rio Blanco Co. 2010 site visits confirmed stem mining activity and progeny adults, indicating successful establishment. In June 2010, new releases utilizing, in part, adult weevils from the 2009 release were made at three additional sites in Rio Blanco Co. (two caged and one open releases) and at one site in Eagle Co. (caged release). All releases will be monitored in 2011 to document establishment and assess weevil population size.

Rearing the yellow toadflax-adapted 'strain' of *Mecinus janthinus*. Colorado State U. partners conducted a greenhouse-based rearing program for *M. janthinus* in 2009 and 2010 (FCL 2009 Annual Report) developing effective rearing protocols for the weevil and producing more than 800 *M. janthinus* adults currently in cold storage. Both products will be used in an FCL rearing effort, which was initiated in late 2010. In 2011, we will scale up *M. janthinus* rearing on yellow toadflax, providing adults for field release in late 2011 (dormant adults in stems) or in spring 2012 (active adults). Field collections from Montana sites are not sustainable, since current *M. janthinus* populations are small and constrained by weevil-caused yellow toadflax mortality.

Biological Control of Tropical Soda Apple in Florida, Georgia, and Alabama using the Leaf-feeding Beetle, *Gratiana boliviana*

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Tropical soda apple (TSA), *Solanum viarum*, is an invasive perennial shrub native to South America that belongs to the plant family Solanaceae. Records indicate that this plant was initially detected in Glades County (southeast Florida) in 1988. TSA primarily invades pastures, fields, and parks, but also has the potential to invade open forest and other natural areas. TSA forms thick stands that can be impenetrable to livestock, large wildlife, and humans. Livestock, deer, raccoons, and other wildlife eat the mature fruit and spread the seed in their manure. TSA also serves as a reservoir for various diseases and insect pests of solanaceous crops. Annual control costs have been estimated at $6.5-16 million. It was placed on the Federal Noxious Weed List in 1995, and it is listed as one of the most invasive species in Florida by the Florida Exotic Pest Plant Council.

PPQ-ER began supporting efforts to develop and implement classical biological control for TSA in 2002 that were underway at the University of Florida-Gainesville, with CPHST providing technical oversight of the project. A permit to field release the South American leaf-feeding beetle *Gratiana boliviana* was approved in 2003. The beetle (**Fig 1**) has since been introduced into Florida (2003), Alabama and Georgia (2004), and Texas (2007) through a collaborative effort by individuals from university, state and federal agencies in these four states (see cooperators listed above), who developed methods to mass rear (Chong et al. 2009), release and evaluate the biological control agent. More than 200,000 beetles have been released in over 350 locations. This technology was transferred through trade and scientific journal publications and YouTube videos to extension agents, cattle ranchers, conservation organizations, and others as part of an integrated management approach to controlling TSA. A multi-agency, state-wide survey in Florida was conducted in 2009 to determine the distribution of *G. boliviana*. The survey revealed that the beetle was spreading on its own and that no further releases were required in south and central Florida, but that it was not well established in north Florida (Overholt et al. 2009). In pastures and natural areas in central and south Florida

Figure 1. *Gratiana boliviana* adults (top photo) and larva (bottom photo) feeding on tropical soda apple.

the beetle has resulted in substantial suppression of TSA density and fruit production (Medal et al. 2010). This was corroborated in a preliminary survey/economic analysis conducted by FAMU in 2010 that indicated in many of these areas ranchers no longer consider the weed a pest and cost for control has decreased significantly.

In Alabama and Georgia, CPHST Miami and ARS Tallahassee collaborated to determine if the beetle could overwinter in these more northern climates and if field insectaries could be established. Although the studies showed that the beetle technically can survive winters in GA and AL, the data generally paralleled those from Florida that indicated the beetle is having less of an impact in northern counties. For example, at the most northern location near Montgomery, AL, the beetles survived through two winters that had periodic snow falls. However, the population remained very low and eventually disappeared. Similarly, *G. boliviana* was found to survive but not thrive in field cages in east Texas, where winter temperatures reached the low 30s. At this point it is unclear if poor performance by the beetle in more northern climates is because the beetle does less well in cooler climates, the plant does less well in cooler climates, or because of more aggressive management practices in the north to eradicate the plant. The group (**Fig.** 2) will conduct controlled field studies in 2011 at three different latitudes in Florida to try and determine what factors are limiting *G. boliviana's* success.

CPHST Miami also worked with University of Florida scientists to test the host range of a second leaf feeding beetle *Gratiana graminae*, which is a more low temperature tolerant biological control agent of TSA. In quarantine studies, the beetle fed and developed on Thai variety egg plant (*Solanum melangona*) and wild turkey berry (*Solanum torvum*), as well as TSA. This information was provided to the Technical Advisory Group for Biological Control Agents of Weeds to help determine the risk of releasing this biological control agent in the field.

Outcomes:

Awards

2010 The Florida Entomological Society Achievement Award for Research Teams

YouTube Videos

Identifying Tropical Soda Apple

Releasing *Gratiana boliviana*: A Tropical Soda Apple Biological Control Agent

Tropical Soda Apple Biological Control with *Gratiana boliviana* - Time Lapse

Tropical Soda Apple: A Rancher's Perspective

What To Expect When Using Tropical Soda Apple Control Agents

Figure 2. TSA biological control team assessing the impact of the program.

Survey for White Rust (*Albugo candida*) and Other Natural Enemies of Perennial Pepperweed (*Lepidium latifolium*)

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Perennial pepperweed, an introduced mustard (family Brassicaceae) from southeast Europe and Asia, is invasive throughout the western United States. It can establish in a wide range of habitats and can rapidly form large, dense stands that displace desirable vegetation.

Current studies focus on herbicides for pepperweed control. Several postemergent herbicides control perennial pepperweed, but multiple applications are common over several years to treat resprouting shoots and seedlings. Although there is an active program to find biological controls for perennial pepperweed, there are currently none available. Because it is in the same plant family as mustard and canola, there is concern that a biocontrol insect or pathogen would attack an agricultural crop.

Figure 1. White rust pustules on perennial pepperweed.

A white rust disease, characterized by white pustules (**Fig. 1**) containing sporangia on the underside of the leaves, has been identified on perennial pepperweed plants across the United States, especially during wet years. Some reports state that this *Albugo* reduces seed set and number while others report that this white rust provides little or no control.

The primary goal of this project was to evaluate the efficacy of white rust on perennial pepperweed and to survey for other endemic pathogens of the weed in Colorado and Wyoming at a number of field sites. Determining which natural enemies are already present in the United States and assessing their current and potential impact on the target weed is a logical first step in developing biological control as a viable management option. Of the eleven races of *Albugo candida* reported to cause white rust on a range of hosts within the Brassicaceae in North America, there have not been reports on which race causes white rust of pepperweed. A second goal of this project involved a race determination of the *Albugo candida* on pepperweed and comparison with the races that cause disease on mustard and canola using a host differential.

CPHST Ft. Collins, in collaboration the University of California, collected isolates of *Albugo candida* from perennial pepperweed in Colorado and California. Results of the host differential inoculation studies indicated that CO and CA isolates of the pathogen may constitute a new race because they did not conform with results for the 11 known races of *A. candida* occurring on weeds and crop species in the United States.

Native and invasive *Lepidium* species, crop species, and other species within the Brassicacae were grown to begin the preliminary stages of host specificity testing for this pathogen in 2010. Both CO and CA isolates showed a slightly different, narrow host range. The Colorado isolate infected four hosts in addition to perennial pepperweed; *Iberis umbellata*, *L. ruderale*, *L. sativum*, and *Stanleya pinnata.* The California isolate infected these four hosts but also caused disease in *L. campestre*, *L. draba*, and *Thelypodium integrifolium*.

ITS sequencing is currently being conducted on the isolates from California and Colorado and a disease report to be published in Plant Disease is in progress.

Biological Control of *Sirex noctilio* in North America by *Beddingia siricidicola*

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Evaluating the effectiveness of the Australian "Kamona strain" nematode as a biological control agent of *Sirex noctilio* necessitates distinguishing it from the conspecific "North American strain" already present in the U.S. Evidence collected this year suggests that the North American strain generally has lower rates of Sirex egg sterilization than does the more effective Kamona strain. Our molecular analyses have found differences of two base pairs, by which strains can be identified. Additionally, there is a strong possibility that the two strains will hybridize in the field, confounding identification. Accordingly, reciprocal crosses were made this year that will be used to develop hybrid cultures for DNA sequencing. "Controlled releases" (that is, with inoculated trees sampled and chipped before Sirex emergence) were carried out in the field to compare the infection rates of the Kamona and North American strains as well as the parasitism rate of the parasitic wasp, *Ibalia leucospoides*, in different stand types. Of the four experimental stands in 2010, the stand in Pennsylvania had red pine whereas the three in New York contained Scots pine. The red pine stand had significantly higher Sirex density than the Scots pine stands, but had the lowest nematode infection rate, where rates ranged from 2% to 35% overall. Stand percent parasitism by Ibalia ranged from 5% to 21% but did not differ significantly among stands. One large Scots pine stand had a higher infection rate (albeit not significantly so) in nematode-inoculated trees than in uninoculated controls. The red pine stand had no nematode infection among the uninoculated control trees, suggesting that North American strain nematodes have not yet arrived in northern Pennsylvania. Red pines inoculated with Kamona nematodes exhibited a lower infection rate (2%) than did those with North American nematodes (9%), although the latter nematodes did not sterilize any Sirex eggs. We also continued with laboratory experiments to optimize mass rearing of nematodes, including the effect of the Amylostereum fungus strain and duration of rearing on nematode yields. Slower growing fungal strains generally produced more nematodes in the end than did faster ones. Rearing at room temperature for six to eight weeks provided satisfactory nematode yields for our releases.

Biological Control of Emerald Ash Borer (*Agrilus planipennis*)

LOCATION: Otis Lab
CPHST STAFF: Juli Gould (Lead), Tracy Ayer, David Williams, Ivich Fraser, and Vic Mastro.
CHAMPIONS: Philip Bell (PPQ-ER); Paul Chaloux (PPQ-EDP)
COOPERATORS: Leah Bauer (USFS), Jian Duan (USDA-ARS), John Vandenberg (USAD-ARS), Jon Lelito (PPQ-ER), Zhong-qi Yang, Xiao-yi Wang, Hai-Poong Lee, Galina Yurchenko
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CPHST Otis has been cooperating with scientists at USDA Forest Service and the Agricultural Research Service to develop biological control options for the emerald ash borer (EAB). As of December 2010, EAB has been found in 15 states, and new infestations continue to be found. Classical biological control, the release of natural enemies from the pest's native range, is being implemented as a strategy to control EAB populations. Three species of stingless wasps (parasitoids) from China have been approved for release against EAB. Two species attack EAB larvae (*Spathius agrili* (**Fig.** 1) and *Tetrastichus planipennisi*) and one species attacks EAB eggs (*Oobius agrili*). The larval parasitoids spend the winter as larvae or pupae inside an EAB gallery.

Figure 1. *Spathius agrili,* a larval parasitoid of Emerald Ash Borer.

Releases were initiated in 2007, but in that year and the following, small numbers were released due to rearing constraints. Many more parasitoids (over 33,000) were released in MI and OH in 2009, and sampling to record field reproduction and overwintering took place in the spring of 2010. The first step in assessing the success of parasitoid releases is determining the ability of each species to overwinter. Given the cryptic habitat of EAB eggs and larvae, detecting the presence of parasitoids is difficult and generally requires destructive sampling methods. In early spring the year following parasitoid releases, we sampled ash trees near the release epicenter of selected study sites to assess overwintering. Each tree was cut into sections and debarked, EAB larvae were collected, and parasitoids were reared to the adult stage for identification. *Oobius agrili,* the egg parasitoid, was reared from ash bark samples. We determined that all three parasitoid species could successfully overwinter at study sites in Michigan and Ohio (**Fig 2**). It is important, however, to distinguish overwintering (recovery one year after release) from actual establishment (recovery two or more years after the last release).

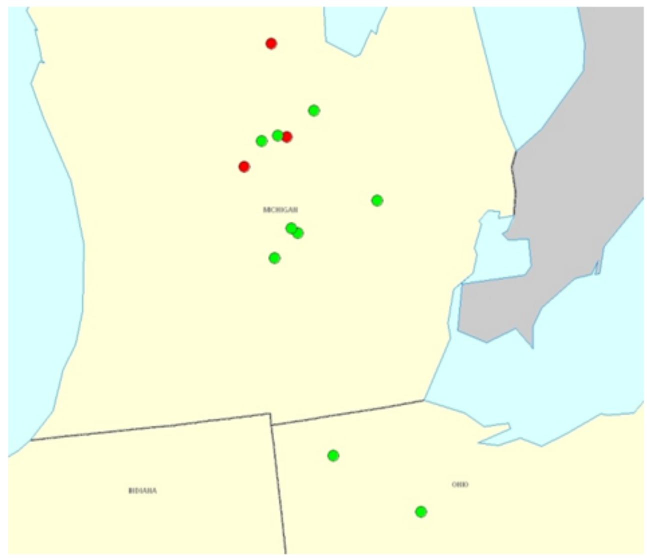

Figure 2. Recovery of EAB Parasitoids in MI and OH.
● = parasitoids recovered; ● = parasitoids not recovered.

Documenting parasitoid overwintering is the first step in evaluating the success of the EAB biocontrol project. The next steps will be to verify establishment and impact on ash health. We will continue monitoring release sites for parasitoid establishment and spread. Methods are being developed to evaluate the impact of parasitoids on EAB population density using life table analyses and indirect evidence of EAB activity. Because EAB density is difficult to determine without destructive sampling, we use indirect evidence of EAB population size at release and nearby non-release control sites.

Life-table studies of the mortality factors (tree resistance, parasitoids) that control EAB in its native range (China, Russia) have been initiated and will be compared to results at sites in the United States. The studies are being conducted in collaboration with ARS and Chinese and Russian scientists. In the native range of EAB, mortality from tree resistance seems higher than in the United States, but mortality from parasitoids (*Tetrastichus* and *Oobius* in China and *Spathius* in Russia) is also considerable.

Studies to develop methods to enhance the probability of parasitoid establishment have also begun. Girdling release trees to attract EAB and providing food (sugar water) for adults are being tested.

Laboratory studies are being conducted to determine if parasitoids released in the fall need to be pre-conditioned to produce progeny that diapause and to determine how late in the fall one can release and still have enough warm weather left for the parasitoids to complete development.

In 2010, CPHST Otis discovered new natural enemies of EAB in South Korea and the Russian Far East, including possibly new species of *Spathius, Tetrastichus,* and *Atanycolus*. These species are from colder climates and might provide superior control in the more northern parts of the U.S. and Canada. Rearing studies and host range testing of the new parasitoids have been initiated and are ongoing.

Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project, 2010 (*Pseudacteon tricuspis, P. curvatus,* and *P. obtusus*)

LOCATION: Gulfport Lab
CPHST STAFF: Anne-Marie Callcott (Lead)
CHAMPIONS: Charles Brown (EDP), Ron Weeks (ER), Shaharra Usnick (WR)
COOPERATORS: Sanford Porter (ARS CMAVE), George Schneider and staff at FL DPI, and State Department of Agriculture and their designees
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The goal of the IFA biological control program is to release and establish 5-6 species of phorid flies throughout IFA infested states. Phorid flies will not be a stand-alone management strategy. A homeowner will not be able to release a few flies in their back yard and see a significant decrease in IFA mounds. Hopefully, however, the flies will be an important tool in integrated approach to IFA management. The added stress caused by these flies on the IFA colonies will allow native ants to compete better for food and territory. This fly-native ant-IFA interaction will hopefully allow homeowners, municipalities and others, to make fewer chemical control product applications annually to suppress the IFA to acceptable tolerance levels, lessening the impact of the IFA on humans, livestock, wildlife and the environment. USDA, APHIS, PPQ began funding a cooperative project to rear and release these biological control agents in 2001.

Preliminary research and rearing techniques have been developed by USDA, ARS for four species, with others under development. ARS will continue to evaluate other phorid fly species for potential use in the U.S., and transfer rearing techniques to the rearing facility as the new species are ready for mass rearing. Mass rearing and shipping of the flies to cooperators is being conducted by the Florida Department of Agriculture, Dept. of Plant Industries (DPI), in Gainesville, FL. CPHST Gulfport provides overall program coordination, lines up cooperators to make and monitor releases, and manages and analyzes program data.

Rearing data: Rearing was initiated in 2001 for *P. tricuspis*, seeded by flies from the ARS-CMAVE facility. The number of rearing boxes in *P. tricuspis* production has increased from the initial 1-2 boxes in 2001 to a high of ca. 10-12 boxes in 2003. Rearing of *P. tricuspis* was at its peak in 2003 and 2004 with ca. 1.6 million flies being produced annually with production gradually decreased to allow increased production of the *P. curvatus* and *P. obtusus* flies. *P. tricuspis* will continue to be reared through 2011 in limited quantities with the aim of eliminating this species totally in 2012. *P. curvatus* rearing was initiated in late 2002, with 1-2 boxes again seeded by flies from the ARS-CMAVE facility. Production of this species was at its peak in 2006 and 2007 with 7 boxes in production and has subsequently decreased as *P. obtusus* production increased. In 2006, the third species, *P. obtusus,* was brought into production. Production has gone well and the first releases of this species were conducted in 2008 and 11 releases to date. In 2010, rearing was initiated on the fourth species, *P. cultellatus*, with the first releases anticipated in 2011. Except for 2009 when production levels were above 3,000,000, total fly production levels have remained fairly constant in the last several years (**Table 1**).

Release data: While flies have been and will continue to be released by various research agencies, including ARS, in many states for research purposes, the goal of this project is to release flies in all federally quarantined states, and ultimately in all infested states. Releases are being coordinated through state plant regulatory officials, with a variety of state groups cooperating with the release and monitoring of the flies.

Releases began in spring 2002. In most cases, the cooperator made the release at one site, however, in a few cases the cooperator split the release and released flies at more than one site. Also, there are several sites were multiple releases over several years have occurred. From 2002 through 2010 there have been multiple releases in each of 13 states and Puerto Rico, with a total of 117 field releases and more than 1,000,000 flies released. Of these 117 releases, 67 were *P. tricuspis,* 39 were *P. curvatus* and 11 were *P. obtusus* (**Table 1**). The average number of potential flies (parasitized ants or pupae) perr release is about 10,000. In 2008, the changing economy had an impact on our cooperators' abilities to conduct releases, and due to lack of resources in many states the number of overall releases in 2008 was less than in previous years. In 2009, we were able to increase our releases from 2008 and have maintained that level through 2010.

In addition to field releases, the equivalent of 3 *P. tricuspis* shipments have gone to Louisiana to seed their own rearing facility, the equivalent of 2 releases have gone to New

Mexico for research purposes, one *P. curvatus* release was abandoned due to site issues, and numerous small numbers of flies have been supplied to cooperators for research or educational purposes, such as state fair exhibits and field days. Louisiana completed its first release from LA-reared flies in 2005, conducted a few releases and then abandoned rearing flies in 2006-2007 and is now releasing APHIS reared flies only. Over 225,000 potential flies have been shipped for these varied uses. A publication on the known U.S.-wide distribution of *P. tricuspis* and *P. curvatus* is currently being reviewed for publication in the Journal of Insect Science with publication expected in early 2011. Maps are currently being developed for this publication.

Table 1. Rearing and release data for APHIS phorid fly rearing project – all species combined (*P. tricuspis, P. curvatus, P. obtusus, P. cultellatus*).

Species	Year	No. flies produced	Approx. no. shipped*	No. field releases**	Mean flies/ release
tri,cur	2002†	950,063	58,750	12	4,895.83
tri,cur	2003	1,746,383	81,450	15	5,430.00
tri,cur	2004	2,280,039	128,602	12	10,716.83
tri,cur	2005	2,765,291	179,813	17	10,577.24
tri,cur,obt	2006††	2,448,798	178,259	17	10,485.82
tri,cur,obt	2007††	2,614,655	137,381	12	11,448.42
tri,cur,obt	2008	2,524,047	80,813	8	10,101.63
tri,cur,obt	2009	3,335,019	88,109	12	7,342.42
tri,cur,obt,cul	2010†††	2,571,357	76,221	12	6,351.75
Total		21,235,652	933,177	117	

* approx. no. potential flies shipped for release

** does not include multiple shipments to LA for initiating their own rearing facility and NM for research purposes, nor multiple shipments to cooperators for educational purposes or small research projects as flies were available

*** shipped for all purposes, field release, initiate rearing, education, etc.

† only tricuspis shipped in 2002

†† only tricuspis and curvatus shipped in 2006 and 2007

††† only tricuspis, curvatus and obtusus shipped in 2010

Updates on LBAM, Beneficial Weevil Projects, and Pink Bollworm

LOCATION: Albany Station (Fort Collins Lab)
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Biological Control of Light Brown Apple Moth (LBAM): A joint project with California Department of Food and Agriculture (CDFA) is focused on possible utilization of parasitoids alone or in combination with sterile insect releases for LBAM control. The rearing system for two species of *Trichogramma* (*T. platneri* and *T. fasciatum*) was developed in the Albany, CA laboratory from which commercial colonies may be established (**Fig. 1**).

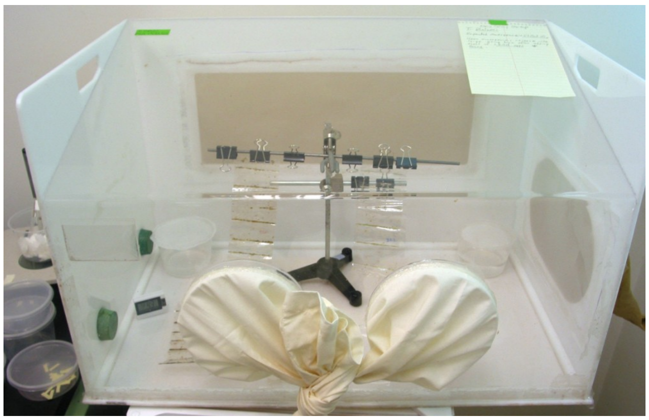

Figure 1. Rearing cage for *Trichogramma* spp. in the lab.

Experimental work in support of *Trichogramma* rearing system development included studies on the effect of different techniques for suppression of LBAM embryogenesis. The objective was to prevent LBAM egg hatching while still providing a suitable host source for parasitism by *Trichogramma*. The methods investigated were UV irradiation and exposure to freezing temperatures. At a 90min exposure period of radiation, only 3% of eggs hatched. Extended exposure times (135, 180 and 240 min) did not further decrease the hatching rate; this may be due to the oviposition behavior of the moth. LBAM lays its eggs in a layered fashion, and the lowest layers of eggs fail to receive sufficient radiation independent to exposure time. Following this, we tested the preference of *T. platneri* on LBAM eggs treated with UV, freezing temperatures (-4^{o}F) or as an untreated control. No significant difference in infestation rate was found between untreated and UV irradiated eggs; those exposed to freezing proved to be undesirable hosts. Irradiation of 90min was accepted as a standard in our rearing procedure. The same procedure is used for egg cards for field studies addressing the seasonality of natural enemies. UV irradiated LBAM eggs were used as a trap host for screening. Identification of captured natural enemies was conducted by CDFA, UC Berkeley and UC Riverside scientists.

Development of rearing systems for beneficial root feeders

Cyphocleonus achetes is a weevil introduced into North America for the control of spotted and diffuse knapweed. Dispersal of *C. achetes* throughout large infestations in the western United States has been very slow due to low numbers available for distribution. With a goal to facilitate the weevil's spread, a rearing system based on an artificial diet was developed. A *C. achetes* colony has been reared in the Albany laboratory for 12 generations on modified artificial diets originally developed for *Hylobius transversovittatus (*a biological control agent of purple loosestrife) and its optimization is progressing. Our work in 2010 was focused on developing a system for storing adult weevils without significantly affecting their reproductive potential. Initial attempts to store *Cyphocleonus* adults under the same conditions as *Hylobius* (50^{o}F and short light) were not successful. We proceeded by experimenting with different environmental conditions and found that long light (16h L: 8h D) suppressed *C. achetes* oviposition at 77ºF. We are now able to store diapausing adults for 10 weeks and then induce reproduction after that storage period.

In 2010 we produced around 800 adult *C. achetes*. Most of them were used in the experiments associated with the optimization of rearing systems and storage work, although a group of 130 individuals was released into the field in Idaho (**Fig. 2**). This field release was undertaken to determine the ability of laboratory reared *C. achetes* to effectively establish in nature for future releases.

Figure 2. Releasing lab reared *C. achetes* onto knapweeds.

Pink bollworm- Addressing the problem of mass reared insects on the calco red dye lacking red coloration.

The presence of pink bollworm (*Pectinophora gossypiella*) adults without red coloration was unexpectedly detected in the field traps in Arizona. One of the hypotheses is that trapped insects are not from the wild unmarked populations, but from mass reared individuals that were able to complete development in the APHIS rearing facility without retaining visible amounts of the Calico red dye that is placed in their larval diet. The light brown apple moth (LBAM) has been used as a model insect in this study because the same phenomenon was observed in our APHIS LBAM colonies (**Fig. 3**).

Figure 3. Uncolored LBAM pre-pupae reared on a diet that includes Calco red dye.

The first study focused on the frequency of uncolored individuals in the LBAM colony and the possible correlation to the sex and/or weight of the larvae. An unexpectedly high 11.3% frequency of uncolored individuals was detected in the Albany colony. Using logistic regression, the presence of red dye was not statistically correlated with sex or weight when males and females were pooled. But when assessed individually for both weight and sex, both of these variables were highly significant (p=.0005 and p=.0020, respectively). So weight affects this frequency differentially between males and females.

To refine the method for determining coloration level in individual insects, an absorption spectroscopy method was developed. Due to its sensitivity, this method may be able to identify red dye in insects that visually appear uncolored. This project is being conducted in cooperation with the Pink Bollworm Rearing Facility in Phoenix, AZ.

Presentations:

-William Roltsch, Nada Carruthers and Richard Stouthamer (2010). Egg parasitisam of the invasive light brown apple moth (*Epiphyas postvittana*) Annual meeting of the Entomological Society of America, December, San Diego.

-Nada Carruthers and David Madieros (2010). Induction of reproductive diapause for prerelease storage of *Cyphocleonus achetes* Coleoptera (Curculionidae). Annual meeting of the Entomological Society of America, December, San Diego.

Biological Control of the Light Brown Apple Moth

LOCATION: Moss Landing, CA
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CHAMPIONS: CDFA, PPQ SPHD-CA, PPQ WR, PPQ EDP
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The sterile insect technique (SIT) has been used to eradicate and control a number of pests in North America, such as Mediterranean and Mexican fruit flies, pink bollworm, screwworm and codling moth. It is now being developed and tested as an option for the light brown apple moth (*Epiphyas postvittana*, LBAM). However, SIT does not have to be a standalone tactic and the full potential for its success may occur when it is combined with other pest suppression methods. SIT with combined releases of egg parasitizing Hymenoptera of the genus *Trichogramma* may provide a synergistic pest suppression method that is more effective than either technique alone.

In SIT release programs for Lepidoptera, both treated males and females are released into the environment. This results in eggs being laid from several different types of crosses involving both irradiated moths and non-irradiated wild moths. Several *Trichogramma* spp. have demonstrated the ability to reproduce on the sterile eggs of irradiated moths. The objective of this research was to assess the ability of *Trichogramma platneri*, a native *Trichogramma* species in CA and one that is available commercially, to suppress LBAM populations alone and in combination with sterile moth releases.

Treatments:

1) Control: 10 pairs of non-irradiated LBAM (10 ♀ and 10 ♂).

2) Parasitoids Only: 10 pairs of non-irradiated LBAM (10 ♀ and 10 ♂) and 330 female *Trichogramma platneri* with a sufficient number of males for mating.

3) SIT Only: 10 pairs of non-irradiated LBAM (10 ♀ and 10 ♂) and 100 ♂ LBAM irradiated at300 Gy.

4) SIT plus Parasitoids: 10 pairs of non-irradiated LBAM (10 ♀ and 10 ♂), 330 female *Trichogramma platneri* with a sufficient number of males for mating, and 100 ♂ LBAM irradiated at 300 Gy.

Field cages (12' x 24' = 288 ft^2) were constructed of 1" galvanized pipe and covered with 52 x 52 sq mesh/inch Lumite Saran insect screens (**Figs. 1a and 1b**). Each cage contained 10 host plants – 5 each of wax myrtle (*Myrica californica*) in 1 gal. containers and red-tipped *Photinia* (*Photinia fraseri*) in 5 gal. containers (Figure 1b). Plants were irrigated 3-times per week with micro-irrigation Spot-Spitters®.

Figure 1a.

Figure 1b.

Parasites were released 3 days post moth release. Plants were checked for LBAM egg masses 11 days post moth release. A subsample of parasitized egg masses were removed and brought to the lab. These were reared as voucher specimens for the *Trichogramma* (**Fig. 2**). At 27 days post moth release, all leaves containing egg masses were removed and brought back to the lab and evaluated for egg development and egg parasitism. (**Fig. 3**).

Figure 2.

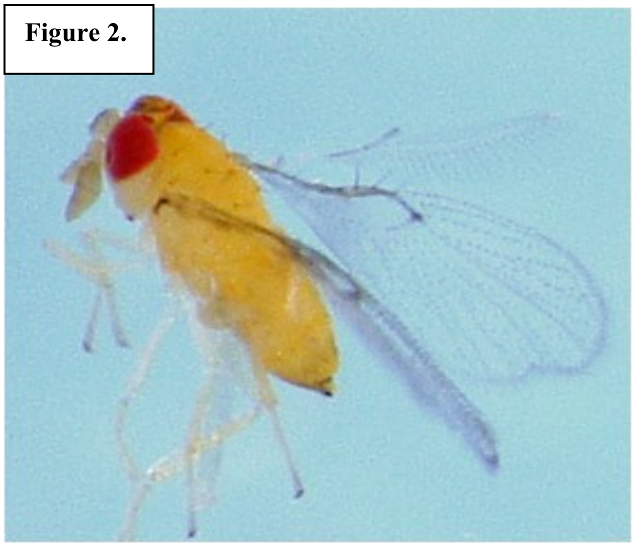
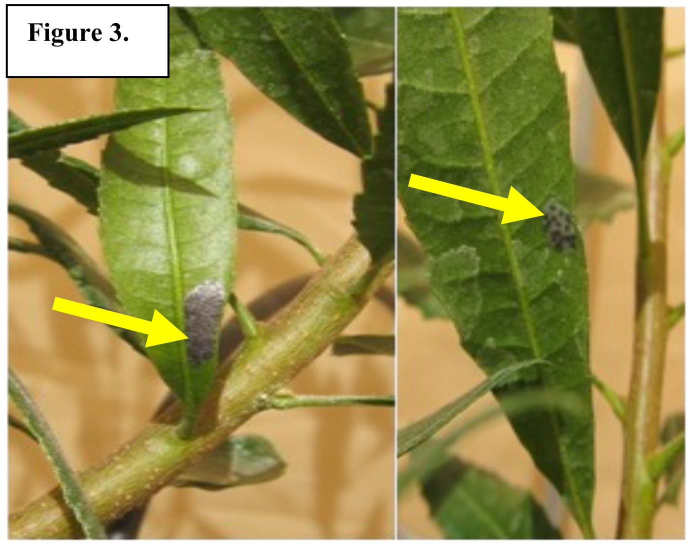


Figure 3.



Results

The high eclosion rate (92%) in the control cage indicates beginning moth quality was high in this trial. The introduction of irradiated moths in the SIT treatment cages reduced the eclosion rate to 16%, indicating most of the eggs were the result of sterile matings (a 76% reduction compared to the control treatment). *Trichogramma platneri* successfully parasitized eggs alone and in combination with sterile moth releases. Over 50% of the eggs laid in the cages with *Trichogramma* were parasitized and LBAM eclosion was $\leq$ 2% (**Table 1**).

These results demonstrate that SIT can be an effective method for control of LBAM. Furthermore, the inclusion of *Trichogramma platneri* combined with SIT could be a useful method for suppression of LBAM in infested areas. The results from this experiment are promising, and indicate the methods used can provide useful data in assessing the impact of SIT on LBAM suppression and the use of parasitoids in combination with SIT. Future studies will further assess the effectiveness of these two control tactics for LBAM.

Table 1. Summary data of caged field trials evaluating the use of SIT and the egg parasitoid *Trichogramma platneri* for suppression of the light brown apple moth.

Treatment	# Egg Masses Observed	Total Eggs	% Eclosion	% Dead Developed	% Dead Undeveloped	% Parasitism	% Parasite Emergence
Control	3	251	92 ± 6	7 ± 6	2 ± 1	0 ± 0	0 ± 0
Parasite	6	73	0 ± 0	0 ± 0	48 ± 17	52 ± 17	17 ± 14
SIT	22	949	16 ± 7	21 ± 5	63 ± 8	0 ± 0	0 ± 0
SIT/ Parasite	15	646	2 ± 1	13 ± 7	10 ± 5	74 ± 10	74 ± 9

Field Evaluation of One Commercial and Two Newly-Discovered Strains of *Metarhizium* Applied by Ground Equipment Against Grasshoppers on Rangeland in Northern Utah

LOCATION: Phoenix Station (Fort Collins Lab)
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COOPERATORS: Donald R. Roberts, Chad A. Keyser, Everton K. K. Fernandes, Scott Treat (Utah State University), and Stefan T. Jaronski (ARS-NRARL)
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Introduction

Grasshopper and Mormon cricket outbreaks can cause such damage that cooperative efforts may be required for relief. USDA programs that combat these pests currently rely exclusively on traditional insecticide sprays and/or baits. It is not uncommon for environmentally sensitive situations to exist in control program areas and to preclude traditional chemical insecticide treatments; their presence at best complicates (and more often prevents) much needed local or area-wide treatments on rangeland. The development of non-chemical measures has long been desired as an alternative to traditional pesticides for these control efforts; those currently registered for use against grasshoppers have not gained commercial acceptance. The fungus *Metarhizium acridum,* active against and specific to Orthopterans, has been found in Australia, Africa, Brazil, Peru, and Mexico and is registered for use in several countries. However, it has not been found in the US, nor have those foreign strains been registered for use here.

Another fungus, *M. brunneum* (formerly *M. anisopliae*) Strain F52, has been registered in the U.S. for control of Coleoptera and for soft bodied ticks (Novozymes Biologicals, Salem VA). In laboratory bioassays with immature Mormon crickets, we observed that the F52 strain was highly infectious, presenting a potential alternative.

The most popular belief concerning ineffective results using *B. bassiana* (another fungus registered for grasshoppers but not generally accepted for use) against unconfined field populations of grasshoppers supposes that infected insects increase body temperatures higher than their typical levels by increasing their exposure to sun, resulting in a body temperature too high for fungal growth. Additionally, after sundown body temperatures may drop too low for fungal growth. This thermoregulation by the grasshopper yields only a few hours each day during which fungi can develop. As a result, the mycosis takes too long to significantly reduce grasshopper populations in a timely fashion. Simply put: For a fungi to work as a control agent the strain must be active against the target pest and the also must be able to replicate at the host pest temperatures in a timely period.

Scientists from CPHST Phoenix Lab, USDA ARS-Northern Plains Agricultural Research Laboratory (NPARL), and Utah State University (USU) have been collaborating to better understand the parameters under which the native fungi *B. bassiana* and *M. brunneum* show useful activity against grasshoppers or Mormon crickets. That effort has been energized by the major effort of the PPQ Western Region which, in the process of conducting normal grasshopper surveys, collects and ships soil samples to USU for isolation and identification of potential fungal pathogens. This could yield a domestic strain of *M. acridum* and/or additional fungal strains that may be useful against grasshoppers, Mormon crickets and other pests.

The following study used ground application spray equipment to apply several isolates of *Metarhizium* to 10 acre plots of grasshopper infested rangeland.

Objectives

(1) Determine the efficacy of different strains of fungal agents on overall grasshopper populations and individual species.
(2) Determine the posttreatment period required until significant initial mortality and final effective mortality.

Methods and Materials

The study site was in Howell Valley, Box Elder Co., near Tremonton UT. The location was selected because of its diversity and density of grasshopper species and grasses and contiguous rangeland suitable for the study. The vegetation was composed of nearly monospecific stands of two dominant plants (sagebrush, *Artemisia triplex confertifolia* and winterfat, *Ceratoides lanata*) and supports a variety of upland game including grouse, pheasant and mule deer.

The DWR346 and DWR356 strains of *M. robertsi* (Donald Roberts, USU) and the F52 strain of *M. brunneum* (Novozyme Biologicals) were each ground applied to a 10 acre rangeland plot. An additional plot treated with only the oil carrier was also included in the design. Each plot was surrounded by a 110 ft. wide treatment of carbaryl (Sevin XLR Plus) to minimize possible grasshopper movement.

Testable quantities of *M. robertsi* were produced by Stefan Jaronski at the USDA-ARS-NPARL in Sidney, MT. The conidia were formulated in the lab in emulsifiable vegetable oil (Golden Pest Natural Oil) at 1 x 10^{12} conidia / quart. Each quart was mixed with 7 quarts of water (two gallons total) for application.

All treatments including the carbaryl buffers were applied using a commercial ground application spray system; Jackrabbit Pro ATV Sprayer (Warne Chemical and Equipment, Rapid City, SD) mounted on a Suzuki Vinson QuadRunner ATV. The spray system was modified to deliver material through a single nozzle Field Jet ¼ KLCSS 5 (Spray Systems) and calibrated to deliver 2 gallons / acre at 6 mph and 22 psi with a 25 ft wide swath.

When we arrived to apply the treatments on 12 July, land adjacent to our study site was being aerially treated with diflubenzuron (Dimilin 2L) by helicopter for rangeland grasshoppers, and that treatment was nearly complete. Our study continued as planned, since it was not feasible to reset the plots. Vegetation samples were collected in areas that may have received drift from the helicopter treatments and sent to CPHST Gulfport to test for diflubenzuron contamination. Our treatments were sprayed before noon 12 July, and the buffer zones were treated with SEVIN® (12 fl. Oz. / acre) the following day.

To assess density, forty 0.1 m^2 rings were placed in four rows (10 rings per row) in the center of each plot. Rows were separated by 30 feet and rings within rows were separated by 15 feet. Grasshoppers were counted on approach by the observer as they jumped from the rings, and each ring was carefully examined to assure that no individuals were missed. Counts were conducted two days before treatment and up to 45 days after treatment.

To determine the species composition at each ring site, two sets of sweep samples were collected. Each sample consisted of 50 high and fast sweeps (older instars and the more active species) and 50 low and slow sweeps (for young instars and less active species). Sweep samples were always collected immediately after grasshopper densities had been determined and were cold stored until they could be sorted and identified. USU completed the data collection, including grasshopper counts and samples.

Results

Data analysis for the first 21 days of the experiment indicate that no significant difference can be seen between plots, including the untreated sites (**Fig. 1**). Only two samples taken to detect diflubenzuron from the unplanned helicopter spray detected this chemical. Our test plots were apparently uncontaminated.

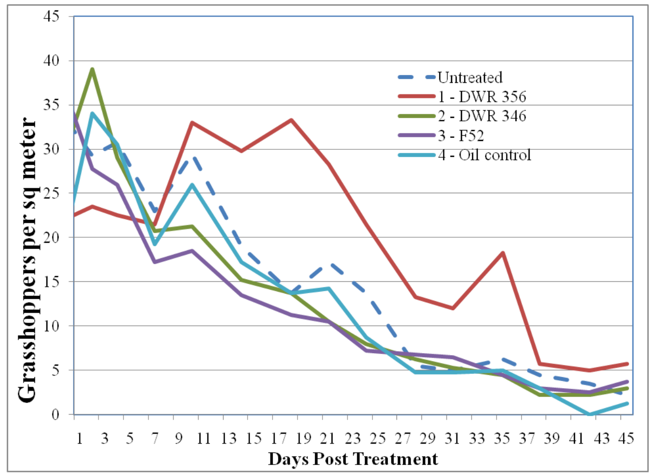

Figure 1. Population trends among the plots treated with the biopesticides and their associated controls, Howell Valley, UT, 12 July through 26 August, 2010.

Several factors potentially confounded our results:
-Movement of grasshoppers into and out of plots.
-Plot elevation; grasshoppers increased in the DWR 356 plot while declining in the others. This plot had the lowest elevation and vegetation may have had better access to ground water and moist air. Grasshoppers may also have been dispersing to the plots from a roadside margin.

-High populations of spiders. While spiders are commonly seen on rangeland, in our experience populations of the size we observed in this study are highly usual. Most individuals were large and formed wide above ground webs which were trapping large numbers of grasshoppers.

Conclusions

Although we did not find significant difference among treatments, we gained valuable experience with the application technology and the temperature issues associated with the biopesticides. Future studies will focus on more optimal target age structures, environmental temperatures and isolates with more attractive thermal windows of activity that are consistent with typical conditions in the western US.

Acknowledgements

Thanks to: Lisa Mosser, Senior Analytical Chemist, CPHST Gulfport Laboratory for analyzing the grass samples for diflubenzuron; Lori Spears, Dept. of Biology and Ecology, USU, for identifying the spiders captured during the study.

Red Palm Weevil

LOCATION: Miami Station
CPHST STAFF: Amy Roda (lead) and Scott Weihman (support)
CHAMPION: Valerie F. DeFeo, PPQ Emergency and Domestic Programs
COOPERATORS: Florida Mechanical and Agricultural University, ARS Gainesville, Department of Agriculture, Husbandry and Fisheries (Aruba and Curacao). APHIS IS (Caribbean Area, Venezuela)
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In tropical and subtropical regions, palms are an important component of the native landscape. Often, they are also economically important plants for agriculture, landscaping, and tourism. In 2009, the Red Palm Weevil (*Rhynchophorus ferrugineus*) (**Fig.1**) was detected in the Caribbean. The pest originates from tropical Asia, but has spread through the Middle-East and the Mediterranean where it has devastated commercial and ornamental palm trees. Damage to palms is mainly caused by the larvae (**Fig. 2**) and only becomes visible long after infection.

Figure 1. Adult red palm weevil

Figure 2. Red palm weevil larvae

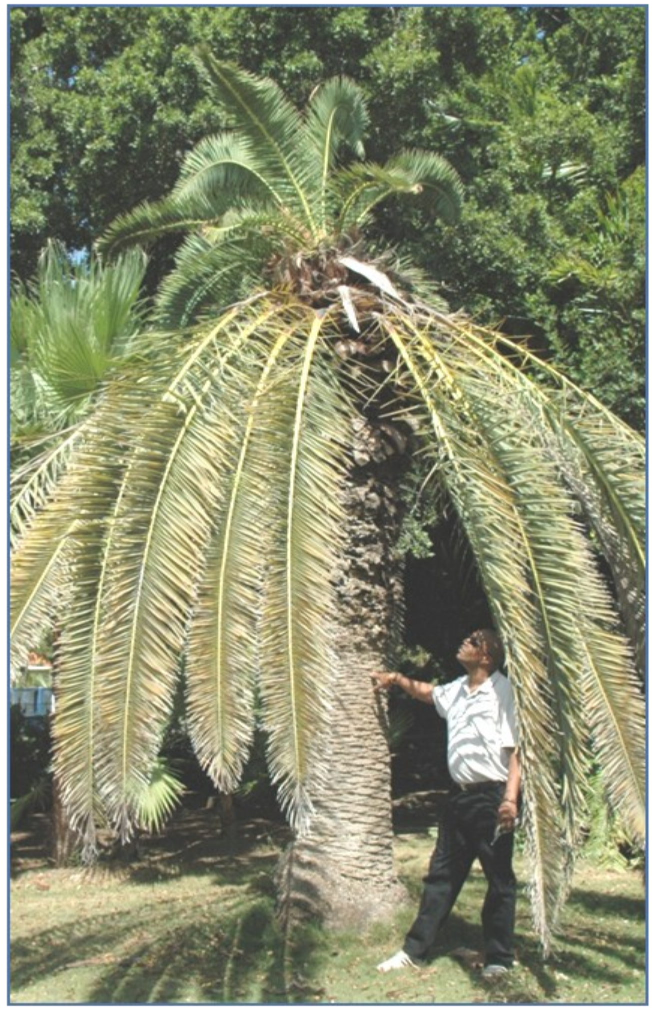

Figure 3. Damage caused by red palm weevil larvae.

By the time the first symptoms of the pest appear, the palms are so seriously injured that they frequently die (**Fig. 3**). Due to the possibility of the weevil spreading through the New World, studies were conducted to determine the extent of the red palm weevil infestation, assess what approaches the government and private entities were taking to manage the pest, optimize survey tactics and protocols, and develop mitigation strategies that would minimize the pest's impact in the region.

One main objective of the efforts in the Caribbean was to develop a standardized, easily implemented trapping protocol for collecting information on red palm weevil distribution, density and population changes. Preliminary trapping was done using male aggregation pheromone (4-methyl-5-nonanol/ 4-methyl-5-nonanone), plant stress odors (ethyl acetate) and food (molasses) baited traps that were distributed throughout the hotel and residential portions of Curacao and Aruba. There were more red palm weevils caught in the traps placed in Curacao (highest 28 weevils/trap/2 weeks) than Aruba (highest 6 weevils/trap/2 weeks). The trap catches in Aruba decrease to 0 weevils/trap/2 weeks and remained consistently low. Many factors could have influenced the difference in numbers, including date of initial infestation, amount of host material available, and management and sanitation efforts.

The potential of utilizing acoustic technology to detect larvae in trees not yet manifesting visual symptoms was tested under the realistic and challenging conditions encountered in Aruba and Curacao, such as very tall trees and a noisy back ground environment. (**Fig. 4**). Being able to detect larvae in palms that are not yet expressing symptoms would greatly aid mitigation efforts by nursery and landscape managers. For instance, the technology would facilitate decisions on whether nursery stock was uninfested and could be moved to new locations or if application of particular management strategies was needed. The technology could also help determine whether or not particular chemical treatments or other management strategies were effective. Overall, the acoustic detection devices appear to be a feasible method to detect the presence or absence of larvae in the urban environments of Aruba and Curacao and would provide a higher level of detection than simple visual inspection. However, additional studies are needed to determine correlations between populations, age, and size of larvae and ability to detect sounds.

Figure 4. Testing acoustic technology to detect the presence of red palm weevil larvae.

If the pest spreads through the Caribbean Basin, the palm and date industry will likely suffer the economic impacts of lost product, increased treatment costs and potential loss of market through regulatory restrictions on exporting from infested areas. Although the palm nursery industry grosses annually over $203 million, the greatest potential economic impact of the red palm weevil in the U.S. would be the destruction of palms in the landscape areas, especially in tourist areas of the southern United States and the territories. The work conducted as well as the information collected to meet the goals of this Farm Bill Project may help lessen the negative impact of the pest. First, the methods developed to survey for the pest were incorporated into the New Pest Response Guidelines for Red Palm Weevil. The guidelines were available to help Federal and State regulatory design a program to respond to the recent (Oct 2010) detection in Laguna Beach, CA. Additionally, the work in Aruba and Curacao helped mobilize local government and private efforts to survey and control the pest. Their efforts to control the pest will help limit the potential that the weevil will spread throughout the region and eventually into the U.S.

Coffee Mealybug (*Planococcus lilacinus)*

LOCATION: Miami Station
CPHST STAFF: Amy Roda (lead) and Scott Weihman (support)
CHAMPIONS: Ron Weeks (ER Regional Program Manager)
COOPERATORS: Florida Mechanical and Agricultural University, APHIS IS (Caribbean Area), Instituto Dominicano de Investigaciones Agropecuarias y Forestales, and Dominican Republic Ministry of Agriculture (Departamento de Sanidad Vegetal)
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The coffee mealybug (*Planococcus lilacinus)* is a plant pest from Asia that attacks at least 96 different species of plants including important agriculture and ornamental crops. In the Western Hemisphere, the mealybug occurs in Hispaniola (Haiti and the Dominican Republic), El Salvador and Guam and is not known to occur in the continental U.S. A recent pest risk assessment has concluded that the likelihood of this pests becoming established in the USA is high and the consequences of their establishment would be severe. Biological control agents could play a significant role in an integrated pest management program. Many pest mealybugs are effectively managed by their natural enemies. Additionally, the extensive host range of the coffee mealybug makes wide scale chemical management unrealistic. Developing biological control technologies for this pest mealybug off-shore will help to reduce the risk of these pests being introduced into the USA, as well as to provide an immediate domestic response capabilities if they should reaches US shores.

CPHST Miami, in cooperation with APHIS-IS Santo Domingo, DR, the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF), Dominican Republic Ministry of Agriculture (Departamento de Sanidad Vegetal) and the Florida Agricultural and Mechanical University (FAMU), initiated a project to determine the impact of the coffee mealybug *Planococcus lilacinus* on local agricultural in the DR and whether natural enemies are providing any control for the pest. The project will form the basis of a Master's thesis for a FAMU graduate student from the Dominican Republic. Working with IDIAF and coffee/cacao grower associations, production areas were identified and initial mealybug surveys were conducted in 19 of the 31 provinces with at least 5 farms were surveyed at 128 locations in order to locate populations of the coffee mealybug. Coffee and cacao plants as well as an array of other host plants were surveyed (**Fig. 1**). A sample of the mealybugs and any natural enemies found associated with them were collected. In the laboratory, a subset of the mealybugs were encapsulated in order to determine if they were parasitized and another subset was separated for identification (**Fig. 2**). Internationally recognized mealybug taxonomy experts

Figure 1. Dr. Colmar Serra, IDIAF (left), Enger German, FAMU graduate student from Dominican Republic student (center), and Dr. Moses Kairo, FAMU (right) look for coffee mealybugs in cacao plantations near Mata Larga, Dominican Republic.

Figure 2. Christopher Cobbs, APHIS IS Agricultural Science Officer, Paula Morales, APHIS IS Agricultural Scientist process mealybugs for identification and to determine parasitism..

have offered to perform the initial identifications. These specimens could then be used as reference collection for the country. A voucher collection of species present in the Dominican Republic is critical to knowing if exotic species are invading the island.

Multiple species of mealybugs were found, however populations of mealybugs were noticeably low. To date, *P. lilacinus* has not been recovered, however, several hundred samples still need to be slide mounted and identified. Already the value of this study has been seen. Of the small proportion of samples identified, the student has already found a new country record: *Hypogeococcus pungens*, which is already established in the U.S. CPHST scientists worked with the student to develop a system to document the apparent low populations that included techniques to quantify the populations as well as taking multiple GPS coordinates. This information, combined with the identification of the mealybugs and natural enemies, could be used to map high risk areas, e.g., those with high mealybug populations, large number of hosts, and minimal management, as well as those areas under good control of natural enemies.

Harrisia Cactus Mealybug

LOCATION: Miami Station and Mission Lab

CPHST STAFF: Amy Roda (lead Miami), Matt Ciomperlik (lead Mission/work in Puerto Rico) and Scott Weihman (support Miami)

CHAMPIONS: Leyinska Wiscovitch (PR SPHD); Ron Weeks (ER Biological Control Program Manager); and Hilda Diaz-Soltero (USDA Sr. Invasive Species Coordinator)

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The Harrisia cactus mealybug (*Hypogeococcus pungens*,) is a severe pest of columnar cacti worldwide. The mealybug also can attack important ornamental plants such as such as Hibiscus, Alternanthera, Acalypha and Portulaca. The mealybug has been reported in Florida since 1984 and has become widely distributed throughout the state. Currently, Harrisia cactus mealybug is not considered a serious pest in Florida, but the mealybug is a major threat to endangered endemic cacti in Pureto Rico and possibly the ornamental and cacti industry in the southwestern U.S. Cacti are highly prized botanical oddities and landscape plants in the U.S. with an estimated value of $6.7 billion combined in Arizona, California, and Texas. The mealybug also presents potential negative impacts to agriculture, precluding the establishment of commercial productions of `dragon fruit', *Hylocereus undatus*.

Figure 1. *Hypogeococcus pungens* (Hemiptera: Pseudococcidae) infesting *Portulaca* sp. in Florida.

The mealybug aggregates on the tips of stems and buds (**Fig. 1**), where its' feeding limits growth and causes distortion. The plants respond by using energy reserves within the tuber system to produce new growth. Eventually the plant dies, as it is unable to support the high continuous energy demands. In Florida, this type of damage has not been reported even though native and ornamental cacti are

Counties	Number of sites visited	Number of positive sites	Total number of cactus mealybugs collected	Percent parasitism
Brevard	4	0		
Broward	5	1	24	45.83%
Charlotte	1	0		
Clay	1	0		
Flagler	1	0		
Highlands	2	1	5	0.8%
Hillsborough	1	0		
Leon	1	0		
Lee	5	2	7 and 75	16 and 42.85%
Manatee	1	0		
MIiami-Dade	9	1	1	0%
Orange	5	0		
Pinellas	8	2	41 and 104	0 and 0.96%
Polk	3	1	1	0%
Seminole	3	0		
St. Lucie	2	0		
Volusia	6	1	23	37.8%

Table 1. The presence and percent parasitism of cactus mealybug, *Hypogeococcus pungens* found in Florida.

commonly found in the landscape. Florida's native biological control fauna could be controlling populations, keeping them below damaging levels.

In order to investigate the impact that natural enemies were having on the mealybug in Florida, CPHST Miami BCU organized and conducted surveys with University of Florida cooperators in areas where the pest was previously collected. To maximize the chance of finding this relatively rare mealybug, the Florida's Division of Plant Industry provided a list of locations were inspectors and extension agents had found the pest. The group surveyed potential host plants including cactus and ornamentals like portulaca, hibiscus and Joseph's coat (Alternanthera spp.). A total of 58 locations in 17 counties were surveyed. Only 9 locations of the 58 sites had very low infestations of *H. pungens*, confirming the 'non-pest' status of the mealybug in Florida (**Table 1**). Interestingly, this cactus mealybug was found only on the ornamental plants and never on cactus.

The study indicated that the mealybug was likely under good biological control in Florida (**Figs. 2 & 3**). Multiple predators and parasitoids were collected and found to be widely distributed across Florida (**Table 2).** All sites were found to have natural enemies except for the two sites where only one mealybug was found (**Table 1**). Based on encapsulation of collected mealybugs, percent parasitism ranged from 0 to over 40%.

The identified predators and parasitoids may work as classical biological control agents against the cactus mealybug in Puerto Rico, where the mealybug is decimating native cactus populations. CPHST Miami BCU will continue to support CPHST Mission's efforts in developing a biological control program by collecting additional natural enemies to determine molecularly if different species are present. If the Florida species are found to be unique, CPHST Miami BCU will develop methods to collect the potential biological control agents for future testing and potential mass production.

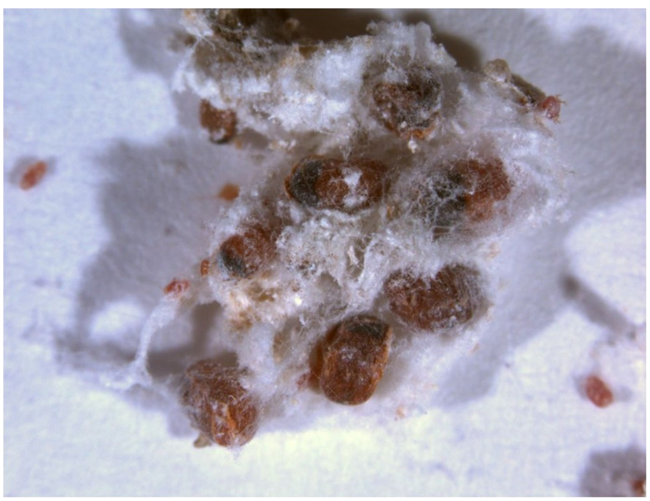

Figure 2. *Hypogeococcus pungens* mummies heavily parasitized by *Leptomastidea* sp. (Hymenoptera: Encyrtidae).

Figure 3. One of two primary parasitoids of *H. pungens* in Florida, *Leptomastidea* sp. (Hymenoptera: Encyrtidae).

Natural Enemy	Order	Family	County Present
Parasitoids			
Gyranusoidea pseudococci	Hymenoptera	Encyrtidae	Broward, Highlands, Lee, Pinellas, Volusia
Leptomastidea sp. Poss. Undescribed	Hymenoptera	Encyrtidae	Volusia
Prochiloneurus dactylopii *	Hymenoptera	Encyrtidae	Volusia
*Aprostocetus sp.**	Hymenoptera	Eulophidae	Highlands
Predators			
Exochomus marginiponnis	Coleoptera	Coccinellidae	Lee
Leucopis	Diptera	Chamaemyiidae	Lee

Table 2. Natural enemies found attacking of Harrisa cactus mealybug, *Hypogeococcus pungens* in Florida.

Biological Control of *Harrisia* Cactus Mealybug in Puerto Rico

LOCATION: Mission Lab
CPHST STAFF: Matthew Ciomperlik
CHAMPION: Leyinska Wiscovitch (PR SPHD
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Figure 1. Galls on *P. royenii* caused by *Harrisia* cactus mealybug.

The Harrisia cactus mealybug, *Hypogeococcus pungens* (Hemiptera: Pseudococcidae) is a recent invasive and destructive pest of columnar cacti in Puerto Rico (**Fig 1**). Surveys since 2005 have found that *H. pungens* is rapidly spreading in Puerto Rican dry forests, and is now distributed in an area of about 1,500 km^2 on the main island (**Fig 2**). It has been responsible for causing significant galling damage and death of affected cacti in 13 municipalities, and will most likely spread to an additional 8 municipalities. Survey activities during 2010 indicate that *H. pungens* is now common in southern Puerto Rico, and is distributed in most dry districts. Heaviest infestations are found in Cabo Rojo Guánica, Lajas, and Salinas. Areas with 100% infestation are common. Fortunately, HCM has not yet been found in Mona, Desecheo, Vieques or Culebra Islands indicating that public education efforts to slow the pests spread seem to be working. Surveys of the affected areas indicate moderate to severe infestations of several key columnar cacti (e.g., *Leptocereus quadricostatus, Pilosocereus royenii, Melocactus intortus, Stenocereus fimbratus* and *Cereus hexagonus*). The affected areas are remote and/or part of the Guanica Dry Forest Preserve; therefore chemical control options are not practical. In this case, classical biological control appears to offer the best management alternative for this pest, hopefully precluding its further spread into the continental U.S.

In 2010, the PPQ Eastern Region and CPHST pooled resources to fund a cooperative agreement with Dr. Alejandro Segarra (UPR, Mayaguez) to: 1) establish and maintain colonies of *H. pungens*, develop and improve on methods for mass production of the mealybug, 2) establish and maintain colonies of *Leptomastidea* sp. (Hymenoptera: Encyrtidae) and *Diadiplosis coccidivora* (Felt)(Diptera: Cecidomyiidae) imported from Barbados in quarantine, 3) and host range testing of both natural enemies to determine suitability for field release to control *Harrisia* cactus mealybug. The activities covered in the cooperative agreement will determine the biology, rearing methods, and host range of potential exotic natural enemies, as required under NAPPO RSPM 12, that could potentially provide a means for controlling HCM populations in Puerto Rico and the mainland U.S.

Figure 2. Municipalities affected by *Harrisia* cactus mealybug in Puerto Rico.

Colonies of both natural enemies were established in the quarantine facility located in Mayaguez, PR. However, early colonies failed to reach sufficient strength and viability to afford studies on the natural enemies biologies. Efforts to develop mass rearing methods for HCM continued and met with success when *Alternanthera bettzickiana* Regel (**Fig 3**) and *Portulacca* sp were used as host plant materials.

Figure 3. *Alternantera bettzickiana* infested with HCM used in parasitoid rearing.

HCM immatures collected from infested cacti from the Guanica Dry Forest area were used to infest *Alternanthera* , and HCM readily shifted to the alternative host plant material. At this point, additional shipments of natural enemies from Barbados were not possible, as suitable HCM populations were difficult to find, presumably the natural enemies reduced HCM below detectable levels at locations where collectsion were previously made. In order to continue ongoing research a decision was made to establish a colony of the native *Leptomastidea* nr *antillicola* obtained from field populations found attacking HCM in Puerto Rico.Quarantine rearing efforts for *Diadiplosis coccidivora* did not succeed as the adult midge has a short lifespan under lab conditions, usually surviving less than 24 hrs. Attempts to place *Diadiplosis* adults with host material rapidly upon adult emergence failed to demonstrate either mating or oviposition. Few midges were recovered from the final shipment from Barbados in 2010, so efforts to rear the midge were discontinued.

A graduate student, Emmanuel Vélez in Segarra's lab, evaluated the biology of *Leptomastidea* nr. *antillicola* Dozier (Hymenoptera: Encyrtidae) (**Fig 4**) a parasitoid natural enemy of HCM and reported on methods for rearing both the host and parasitoid in Velez and Segarra (in press). Development times for parasitoid (egg-adult) were 19 to 30 days, with 90% of adults emerging by day 25. Sex ratios in laboratory raised and field collected parasitoids were similar and slightly female-biased, 1.2:1 (females: males). Median male emergence occurred at 21 days, a day earlier than median emergence in females. Velez and Segarra likewise reported on mating behavior of *L.* nr. *antillicola*.

Host range testing has just begun within the quarantine facility at Mayaguez as of August. Cultures of Maconellicoccus hirsutus, Paracoccus marginatus, Planococcus citri, and Planococcus minor have been initiated on their respective host plants, and numbers of individuals are increasing. At this point, cultura maintenance includes removing extraneous predators and parasitoids (mostly Anagyrus) that may impact the host range studies. Once suitable numbers are reached, host range studies will commence.

Cooperators:

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Aixa Ramirez Lluch, Oficina Estatal de Muestreos (PR Dept of Ag), Santurce, PR

Ian Gibbs, Barbados Ministry of Agriculture and Rural Development, Entomology Division, Christ Church, Barbados.

Dr. David Jenkins, USDA ARS Tropical Agriculture Research Station, Mayaguez, PR.

Figure 4. Adult *Leptomatidea*, parasitoid of HCM (photo: Divina Amalin)

Crypticerya genistae Scale, An Invasive Pest in Puerto Rico

LOCATION: Mission Lab
CPHST STAFF: Matthew Ciomperlik
CHAMPION: Leyinska Wiscovitch (PR SPHD)
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The CPHST Mission Laboratory continued its support of the PPQ Puerto Rico Work Unit and the Puerto Rico department of Agriculture in conducting surveys for a new invasive pest scale insect *Crypticerya genistae* (Hemiptera: Monophlebidae), and its natural enemies. PPQ discovered the presence of the scale insect in January of 2007, near a maritime port attacking weedy host plant species including *Mimosa* and *Euphorbia* sp. Surveys of residences and agricultural growing areas in Puerto Rico showed sporadic infestations of the scale insect at moderate to low densities; however, cases of high pest density were noted on pigeon pea (*Cajanus cajan*; Fabaceae) and hot peppers (Capsicum chinense; Solanaceae) (**Fig. 1**). Pigeon peas are an important legume crop of rainfed agriculture in the semiarid tropics, and are widely grown in the Caribbean.

Figure 1. *Crypticyera genistae* scale insects on pigeon pea and hot peppers.

Survey efforts in 2009 and 2010 showed that *C. genistae* spread from its initial infestation of two municipalities to a total of 35 municipalities in Puerto Rico in approximately two years. The pest insect was frequently found on ornamental ground peanut (*Arachis glabrata*; Fabaceae), a common ground cover used in landscaping, and is hypothesized as being a means in which the insect was unintentionally spread. Surveys conducted in 2010 indicated that no significant economic losses were evident in either cultivated crops nor was there evidence of significant impacts to natural environments. Pest populations routinely were low to moderate in most municipalities during the summer and fall seasons when rain is common, but increased to moderate or high levels during the dryer winter and spring seasons. The scale insect populations were generally higher on the south side of the island which is typically more arid, but were readily found in the eastern to northeastern municipalities as well (**Fig. 2**).

Figure 2. Distribution of *Crypticerya genistae* in Puerto Rico.

A small adventive ladybird beetle (*Anovia circumclusa;* Coccinellidae) (**Fig. 3**) has been identified in both Barbados and Florida attacking *C. genistae*, and is thought to play an important role in controlling the pest in those areas. Personnel from the PPQ Puerto Rico Work Unit and the Puerto Rico Department of Agriculture made collections and conducted surveys to determine the presence of extant natural enemies that utilize the new invasive pest as a host. Infested plant material was returned to the laboratory, isolated in plexiglas cages, and individual hosts that appeared

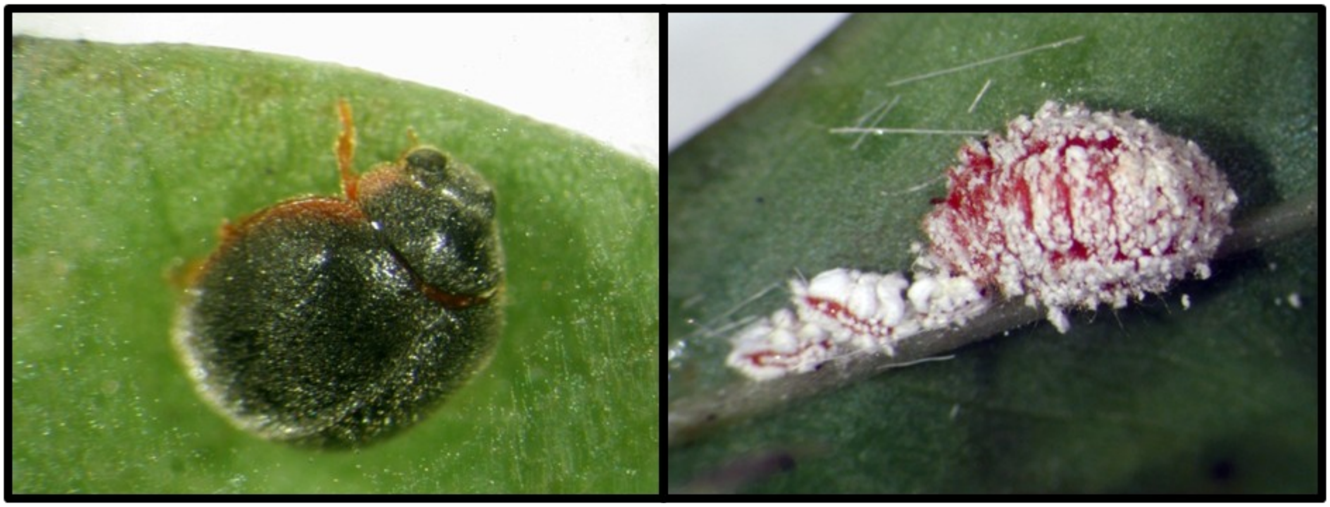

Figure 3. Larva and adult of *Anovia circumclusa* (Coleoptera: Coccinellidae).

to be moribund or showed signs of predation or parasitism were isolated into individual gel capsules. Predators or parasitoids that emerged from the host insect were held for further taxonomic identification. The presence of the lady-bird beetle *A. circumclusa* was confirmed in a majority of the municipalities where the pest was present. In addition, a predaceous phorid fly (*Syneura cocciphila* Coquillett) that is known to commonly feed on cottony cushion scale was recovered from *C. genistae*. The phorid fly was recovered from only a few of the eastern and northeastern municipali-ties (Fajardo, Humacao, etc) in Puerto Rico. Survey efforts in 2011 will continue to monitor the progress of both of the natural enemies to determine their spread and impact on the pest insect.

Neither of the two natural enemies discovered in survey efforts were intentionally introduced. It appears that both predators became established through phoresy along with the scale insect, most likely as immatures either feeding or developing within the elongated ovisac of *C. genistae*. The occurrence of the two natural enemies represents a fortui-tous event that should be documented in the scientific lit-erature, as it has apparently occurred not only in Puerto Rico, but also in Barbados and Florida. Monitoring efforts in Florida and Barbados have shown significant reductions in pest scale prevalence over time due to the impacts of the two natural enemies. Monitoring efforts for both the scale pest and natural enemies should continue in 2011 in Puerto Rico, and traditional biological control efforts (i.e., foreign exploration, quarantine screening, and introductions) should be postponed pending the outcome of those studies.

Collaborators:

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Dr. Alejandro Segarra, University of Puerto Rico, Agricul-tural Experiment Station, Mayaguez, PR

Dr. Divina Amalin, University of Florida, Subtropical Horti-culture Research Station, Miami, FL,

Acknowledgments:

The identification of *Anovia circumclusa* (Coleoptera: Coc-cinellidae) was provided by NataliaVandenberg and identifi-cation of *Syneura cocciphila* (Diptera; Phoridae) was pro-vided by F. Christian Thompson, USDA ARS Systematics Entomology Laboratoiy, Washington, D.C.

Biological Control of Asian Citrus Psyllid

LOCATION: Mission Lab

CPHST STAFF: Daniel Flores (Lead); Norman Barr, Leeda Wood, Joe Martinez, Andrew Parker, Steven Rodriguez, Eustorjio Rivas, and Joe Renteria

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The Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (ACP), is the primary vector of the bacterium 'Candidatus Liberibacter asiaticus' which causes Huanglongbing (HLB), more commonly known as greening disease which is one of the most important diseases of citrus world-wide. The importance of ACP as a pest of citrus derives from its high efficiency in transmitting this bacterial disease, rather than from any direct damage. The ACP has been present in Florida since 1998 and in Texas since 2001. Recently, the vector has started to appear in other citrus-growing regions of the United States as well as parts of Mexico. Citrus greening disease symptoms are described by trees showing stunted growth, sparsely foliated branches, unseasonal bloom, leaf and fruit drop, and twig dieback. The disease was found in Florida in 2005 and is now widespread; HLB has been recently detected in Georgia, South Carolina, and Louisiana, with the most recent detection in the Yucatan Peninsula of Mexico. These detections increase the possibility that the disease may become established in other states where ACP is established and pose a serious threat to citrus production. This past year Mission Laboratory personnel focused on the importation, host-testing, and methods development of mass-production techniques for the biological control of ACP as part of an area-wide management program on citrus.

Foreign Collection of Natural Enemies

Through an interagency agreement with ARS, arrangements were made with Dr. Matt Purcell (USDA-ARS ABCL) to visit China in late April / early May 2010 to make collections of new strains of *Tamarixia radiata*. We provided him with packaging material, shipping permits and labels to begin shipments the first week of May to our Quarantine facility. Coordination was also made with the Los Indios Port of Entry to receive and inspect packages upon arrival into the US and to notify us immediately upon the arrival of insects. Four collections of *T. radiata* were successfully received and established in quarantine.

Importation of Natural Enemies into Quorarntine

Arthropod Quarantine facility is maintaining foreign collections of *T. radiata* (**Fig. 1**) that were made in (1) Lianzhou Town, Guangxi, (2) Yangjiang City, Guangdong, (3) Guangzhou, Guangdong and (4) Hong Kong. In addition, we have a (5) Multan, Pakistan strain of *T. radiata* which was collected in August 2009 and will be undergoing host-specificity testing at the Mission Laboratory alongside the work being done by Dr. Mark Hoddle at University of California-Riverside to meet the requirements of the permitting process. We are maintaining three non-target species of psyllids to test (**Fig. 2**).

Figure 1. A total of five foreign collections of *Tamarixia radiata* have been imported into PPQ's Arthropod Quarantine in Mission, TX. Collections were made by Matt Purcell (China) & Abdul Rehman (Pakistan). Facilitators for importing and receiving were Leeda Wood & Dan Flores.

Figure 2. Host-specificity testing of *Tamarixia radiata* in PPQ's Arthropod Quarantine Laboratory. The test agent was collected and imported from the Punjab region of Pakistan into Quarantine. This strain is of great interest because of its potential to be climatically adaptable to major citrus producing regions affected by ACP. The work is a collaboration between USDA APHIS PPQ CPHST Mission Laboratory, Edinburg, TX and Department of Entomology, University of California Riverside, CA. Mass-production methods are also being developed to have in place when permit for field release is approved.

Mass Production of Natural Enemies

Methods to produce large numbers of *Tamarixia radiata* were tested by releasing an endemic strain of the biological control agents on mature citrus trees infested with ACP inside a field insectary cage. Tree hedging was conducted to induce the growth of new flush. Adult psyllids were attracted to the tender growth and began egg-laying. A field insectary cage was installed around the hedged tree. Five hundred parasitoids were then released into the cage. Two months later, samples were collected and scanned under the microscope to observe parasitism rates. Percent parasitism and total parasitoid production was calculated. With a trial release of 500 adults made during March 2010, it was evident by May 2010 that *T. radiata* could be mass produced in field insectary cages. Parasitism rates were recorded at 19.56% with an estimated total of 21,600 parasitoids produced. Through further testing, this method may continue to prove to be an excellent tool for introducing, releasing, and establishing parasitoids directly in commercial and organic groves to help suppress ACP populations.

Methods to increase mass-production will continue being developed. Further research is being planned to determine how host-feeding impacts production, how to increase parasitism levels, how to easily harvest emerging parasitoids, and how to enhance and monitor production using day-degree models. Upon permit approval, additional strains of *T. radiata* from the Punjab region of Pakistan and from People's Republic of China, currently quarantined, can be mass produced and released leading to establishment and field evaluation.

International Workshop

In February of 2010, we co-hosted the "USDA, APHIS International Workshop on *Tamarixia* Species" held in McAllen, Texas, which included over 25 presentations and 60 attendees from all over the world. A tour of the Asian citrus psyllid project at CHPST Mission Laboratory was also included.

Future Work

Mission Laboratory personnel will continue to assess populations of the Asian citrus psyllid and to observe the impact of the natural enemies on ACP. The methods for accomplishing this work will include: (1) concluding host-specificity testing of *T. radiata* and to submit permit application for field release of foreign collections, (2) importing additional foreign collections of *T. radiata* and/or other natural enemies in late 2010 and 2011, (3) developing mass-rearing methods for ACP and *Tamarixia radiata* on mature citrus trees and under greenhouse conditions with methods for harvesting large quantities of natural enemies, (4) conducting field releases of the parasitoids in the Rio Grande Valley and other affected areas as requested, (5) assessing the biological control impact especially in the urban environment including organic groves and dooryards, and (6) conducting insecticidal assays on the psyllid and the parasitoids for improving IPM strategies.

Biological Control of the Mediterranean Fruit Fly: Parasitoid Rearing at San Miguel Petapa, Guatemala

LOCATION: Guatemala Lab
CPHST STAFF: Pedro Rendon
CHAMPIONS: Terry McGovern (Assistant Regional Director, Guatemala, APHIS-IS) and Wayne Burnett (APHIS Exotic Fruit Fly Director)
CONTACT: Pedro Rendon (Pedro.Rendon@aphis.usda.gov)

Mass-production

Fopius ceratitivorus Wharton is a braconid parasitoid of the Mediterranean fruit fly (= medfly), *Ceratitis capitata* (Wied.), that was collected from Kenya, east Africa. Shipments of tephritid pupae from Kenya were sent to the USDA APHIS/ MOSCAMED quarantine facility at San Miguel Petapa, Guatemala, Central America in 1998. Since then rearing protocols have been developed and several phases of field evaluation have been conducted. A permit for field release of the parasitoid in Guatemala was granted in 2002. Currently, large scale releases of parasitoids combined with sterile medfly releases are being evaluated. In 2009, an average of 121,000 parasitoids/week was made available for field releases during 5 months of the year. During 2010, after the parasitoid colonies became stable in the new facility, an average of 4,000 female parasitoids/hectare/per week was sent to the field from March to August.

Field releases

Field releases of *F. ceratitivorus* were conducted during 2010 for a period of 18 weeks (March to August). Comparative releases were carried out at farms Culpan, El Faro and Andes-Panama. All of these areas are located within the coffee production region of southwest Guatemala. The areas were selected based on the interest of local action program managers to eliminate recurrent medfly detections (**Fig. 1**).

The test design for assessing the impact of the field releases included 3 km^2 plots, one km^2 for each of the following three treatments: (1) sterile insect releases alone (Culpan); (2) sterile insect releases + parasitoids (El Faro); and (3) a control (Andes-Panama) without releases (**Fig. 2**). The aim of the releases was to determine the resulting infestation level and parasitoid presence from collections of

Figure 1: (Left to right) Finca Culpan, Finca Andes-Panama and Finca El Faro, showing location of traps, and ,in the latter, the addition of parasitoid release sites.

coffee berries from the different plots. To be able to conduct ground field releases, a previously designed prototype, a metal-insulated backpack was used to conduct the releases (**Fig. 3**).

The evidence obtained was that no recurrent infestations have been present in the areas where releases were conducted, although few parasitoids were recovered from the release areas (**Fig. 4**). Based on laboratory studies, much of the mortality caused by the parasitoids is due to host probing and feeding. As a result, the local action program has not needed to invest additional resources in the control of these areas, despite the fact that they have historically been considered reservoirs of the pest in previous years.

Figure 2. Release density of sterile males of Mediterranean fruit fly *Ceratitis capitata* (Wied.) over two coffee farms Culpan and El Faro.

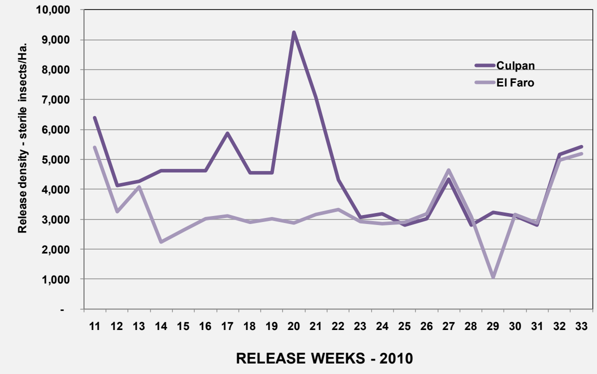
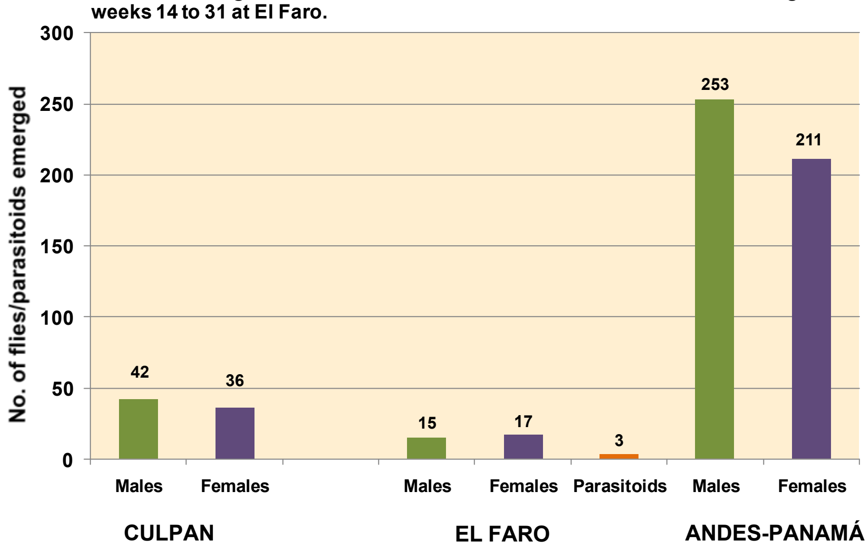


Figure 3: Insulated backpack used for parasitoid ground releases

Figure 4: Infestation level - total number of Mediterranean fruit flies *Ceratitis capitata* (Wied.) and parasitoids *Fopius ceratitivorus* (Wharton) collected from coffee berry samples (120 berries/week/farm). Samples were collected during weeks 14 through to 33, 2010. Parasitoid releases of were carried out during weeks 14 to 31 at El Faro.



The Guatemala Lab Quarantine Facility

CONTACT: Carlos Caceres (Carlos.E.Caceres@aphis.usda.gov)

The Guatemala quarantine facility underwent renovations and expansion in 2010. The size of the original facility (**Fig. 1**) was 931 sq. ft. The expansion added an additional 2,200 ft^2 (32.8 x 67 feet). The total area remodeled includes rooms A to M (**Fig. 2**)

Figure 1. (Right) Original quarantine facility.

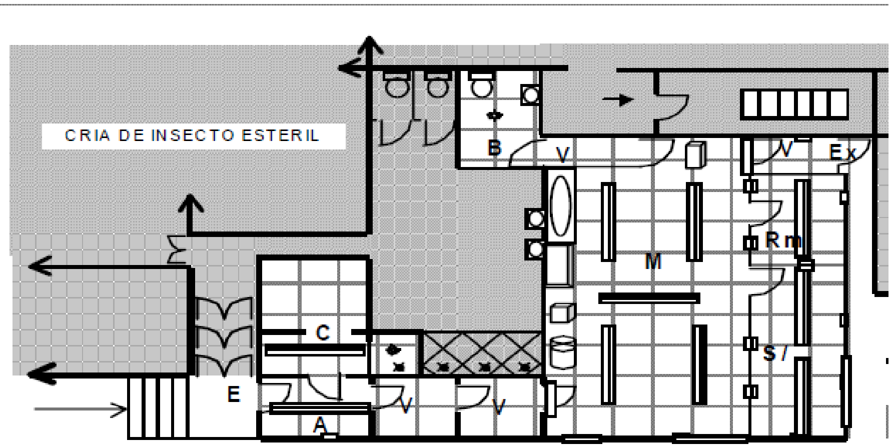


Figure 2. (Below) Quarantine facility after additional 2,200 ft^2 expansion.

The new rooms A through J will be dedicated to rearing and quality control. Area K will house lockers, toilets, and showers. Area L is the new exclusion area and connects the old and new quarantine areas. Area M contains the emergency doors for the new quarantine area.

Main remodeling work included:

- Wall repair and paint
- Installation of new granite floor
- Construction of new concrete false roof
- Installation of a new A/C system
- Drain line remodeling. This will allow treating wasted water before being deposited in septic tanks.
- Installation of new electric line system
- Installation of new lines of tap and hot water
- New set of doors with appropriate insulation

QUARANTINE FACILITY

The new area of the quarantine facility will be dedicated to characterizing new strains of either *Anastrepha ludens* (Mexfly) or Mediterranean fruit fly (Medfly) and the development of rearing technologies for new fruit fly parasitoid species. The quarantine area has sufficient space to produce three million Mexfly or six million Medfly, which will allow us to mimic mass-rearing conditions. The first candidate for assessment will be a black pupal sexing strain of mexfly. A GMO red-fluorescent Medfly strain (from IAEA) is also being examined. A potential future candidate for mass-rearing studies is the olive fruit fly.

Equipment:

Construction of equipment is ongoing. The area will be equipped with new mexfly egging cages, new aluminum pupae racks, and new dollies to transport larval diet trays, and a freezer. Each room will be equipped with essential equipment and instruments for conducting basic QC tests (e.g., stereo microscope, PC, work desk, etc.).

Insect Colonies:

The existing colonies maintained in quarantine are:

- ***Anastrepha ludens* Black Pupal Sexing Strain.**
  Cooperators at the fruit fly genetics laboratory of SAGARPA, Tapachula, Chiapas, Mexico, insolated this genetic sexing strain using classical genetic approaches. In this strain males emerge from normal brown pupae, while females emerge from black pupae. Shipments were made from Tapachula to the Guatemala quarantine facility in May 2010. Evaluations are examining strain stability, strain characterization in mass rearing, male sexual competitiveness and compatibility in field cages and open field, as well as the use of an optical sorter machine to separate (sexes) brown and black pupae. The goal is to replace the current bisexual strain with this new genetic sexing strain to improve the efficiency of Mexfly SIT technology (both rearing and field performance) in ongoing SIT operational programs in the US and Mexico.

- ***Ceratitis capita* Genetically Modified Strain**
  Vienna "8-F307-4M45♀ = 680X:44♂" – Cooperators with Seibersdor Agriculture and Biotechnology Laboratories of FAO/IAEA insolated this GM medfly strain that fluoresce red when observed under a stereo microscope equipped with appropriate filters. Shipments were made from Vienna to the Guatemala quarantine facility in August 2009. Evaluations are looking at strain stability, the persistence of the red fluorescent maker in the field and male sexual competitiveness and compatibility. If successful, transgenic strains with an internal genetic marker could eliminate the use of external fluorescent dyes and act as a primary identification system for operational Medfly SIT programs.

- ***Psytallia cosyrae*** (Hymenoptera: Braconidae) – synovigenic, koinobiont larval-pupal parasitoid of *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) and other tephritid fruit flies, including *C. capitata*. It was originally shipped to Guatemala by ICIPE in June 2002 from Kenya as 200 parasitized fruit fly pupae.

- ***Psytallia nr. concolor*** (Hymenoptera: Braconidae) – larval/pupal parasitoid of olive fly originally collected from Namibia.

- ***Psytallia humilis*** (Hymenoptera: Braconidae) – larval/pupal parasitoid of olive fly originally collected from Kenya.

The Otis Lab Quarantine Facility

CONTACT: Hannah Nadel (hannah.nadel@aphis.usda.gov,)

The 2,500 square foot facility houses three laboratory rooms, three walk-in environmental chambers, a room with reach-in environmental chambers, an area for dressing and dish washing, and one room each for logs, package handling, and gas chromatography. Two autoclaves are used for waste sterilization. Movement in and out of the facility is channeled through a vestibule. In 2010, an air shower was added outside the vestibule to improve security. Following are the pest species in quarantine and primary focus of projects associated with these organisms in 2010:

Asian longhorned beetle (*Anoplophora glabripennis (Motschulsky])* – Insecticide testing, attractant and trap development, host testing, and development of regulatory treatments.

Emerald ash borer (*Agrilus planipennis [Fairmaire])* – Insecticide testing, including biopesticides, parasite evaluation (multiple *Spathius spp.*), production, and trap and lure development.

Sirex woodwasp (*Sirex noctilio F.*) – Development of a phenology model, production of and evaluation of *Beddingia siricidicola* (Bedding) and development of attractants and traps.

Light brown apple moth (*Epiphyas postvittana [Walker]*) – Development of regulatory treatments, and development of management treatments.

European Grapevine Moth (*Lobesia botrana [Denis & Schiffermüller]*) – Development of regulatory treatments, characterization of the radiation biology, development of SIT and of mating disruption formulations.

Asian gypsy moth (*Lymantria dispar [L.] and related species*) – Characterization of flight ability and inheritance of diagnostic molecular markers.

Rosy Moth (*Lymantria mathura* [Moore]) and **Casurina Moth** (*Lymantria xylina* [Swinehoe]) – Characterization of flight ability and response to light.

Winter Moth (*Operophtera brumata [L.]*) – Production and evaluation of parasites *Cyzenis albicans* (Fallén) and *Agrypon flaveolatum* (Gravenhorst).

The Mission Lab Quarantine Facility

CONTACT: Leeda Wood (leeda.a.wood@aphis.usda.gov)

The facility consists of a receiving lab, an identification lab, three laboratories, twelve walk-in environmental growth chambers, three reach-in environmental growth chambers, a headhouse/potting area adjacent to five separately controlled greenhouses, a pot and clothing washing area, storage area and lunchroom. One pass-through autoclave is used for waste sterilization. Movement of personnel in and out of the facility is channeled via pre– and post-entry vestibules through separate men's and women's restrooms/dressing rooms. Entry of biological materials into quarantine and exit of permitted materials out of quarantine is conducted through a secured-access pass-through chamber.

The Arthropod Quarantine (AQ) Operations process was formally documented in the Mission Lab Quality Management System (QMS) and brought into the scope of the lab's ISO registration upon successful completion of a 3rd party audit in March 2010.

Cultures and activities conducted during 2010 in the facility are listed below:n 2010, the facility supported the following

APHIS BIOLOGICAL CONTROL PROGRAMS

Asian citrus psyllid (*Diaphorina citri* (Kuwayama) (Homoptera: Psyllidae)) **-** Receipt of 2 shipments (4 cultures) of the parasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), from China., collected by Australian Biological Control Lab personnel. Host specificity testing and efficacy evaluation of 2 *T. radiata* collections from Pakistan.

Russian knapweed (*Acroptilon repens*) **-** Receipt of 2 shipments (Turkey and Uzbekistan) of the parasitoid, *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) from CABI, Delamont, Switzerland, for quarantine processing and establishment of cultures to support field releases by the CPHST Fort Collins lab.

ARS BIOLOGICAL CONTROL PROGRAMS

Giant reed (*Arundo donax)* **-** Received 11 shipments from Spain (5 of *Tetramesa romana;* 6 of *Rhizaspidiotus donacis*, Arundo scale). The culture of *T. romana* was processed out of AQ upon receipt of the environmental release permit.

OTHER ACTIVITIES

Mexican fruit fly (*Anastrepha ludens*) **-** Maintenance and evaluation of wild cultures of Mexican fruit fly (MFF) for eventual introduction into the MFF mass rearing facility for strain replacement.

Asian citrus psyllid:

- Dense Interplantings of Grapefruit and Guava
- Area-wide Management of Asian Citrus Psyllid

PUBLICATIONS

Peer-Reviewed

Duan, J.J., L.S. Bauer, M.D. Ulyshen, **J.R. Gould**, R. Van Driesche. 2010. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Biological Control 56: 170-174. November.

Duan, J.J., M.D. Ulyshen, L.S. Bauer, **J.R. Gould**, R. Van Driesche. 2010. Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). Environmental Entomology 39: 1513-1522.

Foster, R.N., S.T. Jaronski, K.C. Reuter, L.R. Black, R. Schlothauer. 2010. Explaining mycoinsecticide activity: Poor performance of spray and bait formulations of *Beauveria bassiana* and *Metarhizium brunneum* against Mormon cricket in field cage studies. Journal of Orthoptera Research 19: 303-313.

Fernandes, E.K.K., C.A. Keyser, D.E.N. Rangel, **R.N. Foster**, D.W. Roberts. 2010. CTC medium: A novel dodine-free selective medium for isolating entomopathogenic fungi, especially *Metarhizium acridum*, from soil. Biological Control 54: 197-205.

Medal, J., N. Bustamante, W. Overholt, R. Diaz, P. Stansly, **A. Roda**, D. Amalin, K. Hibbard, R. Gaskalla, B. Sellers, S. Hight and J. Cuda. 2010. Biological Control of Tropical Soda Apple (Solanaceae) in Florida: Post-Release Evaluation. Florida Entomologist, 93(1):130-132.

Yokoyama, V.Y., **C.E. Caceres**, L.P.S. Kuenen, X.-Y. Wang, **P.A. Rendon**, M.W. Johnson, K.M. Daane. 2010. Field performance and fitness of an olive fruit fly parasitoid, *Psyttalia humilis* (Hymenoptera: Braconidae), mass reared on irradiated Medfly. Biological Control 54: 90-99

Wang, X.-Y., Z.-Q. Yang, **J.R. Gould**, H. Wu, J.-H. Ma. 2010. Host-seeking behavior and parasitism by *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer. Biological Control 52: 24-29.

Wang, X.-Y., Z.-Q. Yang, **J.R. Gould**. 2010. Sensilla on the antennae, legs and ovipositor of *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). Microscopy Research and Technique 73: 560-571.

Wang, X-y, Z-q Yang, **J.R. Gould**, Y-n Zhang, G-j Liu and E-S Liu. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in China. Journal of Insect Science 10: 128 (23 pp.).

Yang, Z-Q, X-Y Wang, **J.R. Gould**, R.C. Reardon, Y-N Zhang, G-J Liu, E-S Liu. 2010. Biology and behavior of *Spathius agrili*, a parasitoid of the emerald ash borer, *Agrilus planipennis* in China. Journal of Insect Science 10: 30 (14 pp).

Programmatic or In-house

Bertone, C. P. Michalak, **A. Roda**. 2010. USDA New Pest Response Guidelines. Red Palm Weevil *Rhynchophorus ferrugineus* at: http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprg-redpalmweevil.pdf.

Gould, J.R., L. Bauer, J. Duan, J. Buck. 2010. USDA–APHIS/ARS/FS. Emerald Ash Borer, *Agrilus planipennis* (Fairmaire), Biological Control Release Guidelines. USDA–APHIS–ARS-FS, Riverdale, Maryland.

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Poland, T.M., D.G. McCullough, D. Herms, L.S. Bauer, **J. Gould**, A. Tluczek. 2010. Management tactics for emerald ash borer: chemical and biological control. IN Proceedings of the USDA Interagency Research Forum on Gypsy Moth and other Invasive Species. Annapolis, MD. January 2010.

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Williams, D.W., **V.C. Mastro**. 2010. Adapting nematode-based biological control systems to North America populations of *Sirex noctilio.* IN: Proceedings. 21th U.S. Department of Agriculture Interagency Research Forum on Invasive Species 2010. Gen Tech Rep NRS-P-75 USDA Forest Service, Newtown Square, PA.

Williams, D.W., H.-P. Lee, Y.-S. Jo, G.I. Yurchenko, **V.C. Mastro**. 2010. Exploration for EAB and its natural enemies in South Korea and the Russian Far East 2004-2009. IN: Emerald Ash Borer Research and Technology Development Meeting - 2009, FHTET-2010-01, USDA Forest Service, Morgantown, WV.

COOPERATIVE/INTERAGENCY AGREEMENTS

Biological Control Related Cooperative/Interagency Agreements 2010 Managed by or Partnered with CPHST Scientists

WEEDS:

Target: **Canada thistle**
Title: Identification of eriophyid mites collected from exotic and native *Cirsium* thistles in the western US
Cooperator: Dr. Radmila Petanović, Belgrade Univ., Belgrade, Serbia
Funding: $5,500 – BC Line
ADODR: Rich Hansen

Target: **Exotic Weeds**
Title: Development of weed biological control agents
Cooperator: Ulrich Kuhlmann, CABI Europe-Switzerland, Delémont, Switzerland
Funding: $195,500 – BC Line
ADODR: Richard Hansen
Objective: Pre-release development and host specificity testing of biological control agents of:

Canada thistle	Hound's-tongue
Dyer's woad	Perennial pepperweed
Field bindweed	Orange hawkweed
Garlic mustard	Russian knapweed
Hoary cress	Yellow toadflax

Target: **Hound's Tongue**
Title: Risk assessment and monitoring of target and non-target plant utilization by the hound's tongue root weevil *Mogulones cruciger* in northern Washington and Idaho
Cooperator: Mark Schwarzlaender, University of Idaho, Moscow, ID
Funding: $38,500 – BC Line
ADODR: Richard Hansen

Target: **Russian knapweed**
Title: Rearing of *Jaapiella ivannikovi* and field release in Montana and Wyoming
Cooperator: Dr. Jeff Littlefield, Montana State Univ.
Funding: $5,000 – BC Line
ADODR: Rich Hansen

Target: **Yellow Starthistle, Rush Skeletonweed, Russian Olive and others**
Title: Development of rearing systems for beneficial root and stem feeders
Cooperator: Massimo Cristofaro, BBCA Laboratory, Rome, Italy
Funding: $16,500 – Bureau of Land Management
ADODR: Nada Caruthers
Objective: Testing the use of a general artificial diet developed for root feeding weevils to facilitate field collections while on foreign exploration trips and for rearing larvae of potential agents to adult stage for identification.

Target: **Yellow Toadflax**
Title: Development of rearing techniques for *Mecinus janthinus* on yellow toadflax
Cooperator: Andrew Norton, Colorado State University, Ft. Collins, CO
Funding: $20,000 – BC Line
ADODR: Richard Hansen
Objective: Assess the relative utilization of yellow toadflax vs. Dalmation toadflax by the stem mining weevil *Mecinus janthinus* and develop methods to rear the weevil on caged plants.

INSECTS:

Target: **EAB**
Title: *Spathius agrili* Yang (Braconidae) field release for control of emerald ash borer in Michigan: Non-target effects study
Cooperator: John S. Strazanac, West Virginia University
Funding: $27,563 – EAB Program
ADODR: Juli R. Gould
Objective: Post-release monitoring of the exotic EAB biological control agent *Spathius agrili* for non-target impacts. Three species of wood borers native to North America that were used in laboratory host range testing are the focus of this field study, including the bronze birch borer (*Agrilus anxius* Gory), two-lined chestnut borer (*Agrilus bilineatus* (Weber), and red-headed ash borer (*Neoclytus acuminatus* (F.).

Target: **EAB**
Title: Biocontrol of emerald ash borer: Exploration and research in China
Cooperator: Yang Zhong-qi, Chinese Academy of Forestry, Beijing, China
Funding: $14,030 – EAB Program
ADODR: Juli R. Gould
Objective: Collect and send additional specimens of *Spathius agrili* to increase genetic diversity for mass-rearing and continue investigating natural enemies that attack EAB at low population densities.

Target: **EAB**
Title: Ecology and natural control of *Agrilus planipennis* in South Korea
Cooperator: Professor Hai-Poong Lee, Dongguk University, Seoul, South Korea
Funding: $15,030 – EAB Program
ADODR: David W. Williams
Objective: To identify, collect and study natural enemies of EAB from South Korea.

Target: **EAB**
Title: Monitoring tools for the detection of biocontrol parasitods of EAB
Cooperator: Dr. Allard Cosse', ARS Peoria, IL
Funding: $99,500 – EAB Program
ADODR: Vic Mastro
Objective: To obtain basic information on the chemical ecology of three EAB parasitoids, *Spathius agrili, Tetrastichus planipennis*, and *Oobius agrili*, with the aim to develop identified semiochemicals into practical tools for the detection of the adult parasitoids in the field.

Target: **EAB**
Title: Ecology and natural control of *Agrilus planipennis* in the Russian Far East
Cooperator: Dr. Galina I. Yurchenko, Far Eastern Forestry Research Inst, Khabarovsk, Russia
Funding: $16,030 – EAB Program
ADODR: David W. Williams
Objective: To discover, collect and study natural enemies of EAB from the Russian Far East

(Continued on next page)

Target: **Exotic Invasive Pests**
Title: Development of biological control and other safeguarding tools to manage invasive pests
Cooperator: Moses Kairo, Florida A&M University, Tallahassee, FL
Funding: $200,000 – Office of the APHIS Administrator
ADODR: Ken Bloem
Objective: Six projects projects were supported in 2009, including:
(1) Improving offshore mitigation strategies for invasive pests coming from the Caribbean and Central America (Continuation Project)
(2) Development of protocols for risk assessment and management for agents used in classical and augment tative biological control (Continuation Project)
(3) Economics: An assessment of selected economic aspects of invasive alien species (Continuation Project)
(4) Use of geospatial technologies to understand invasion processes and to mitigate invasive species (Continuation Project)
(5) Assessment of the potential for biological control of Cotton Seed Bug, *Oxycarenus hyalinipennis* (New Project)
(6) Development of an Integrated Pest Management Strategy for the Invasive Small Hive Beetle, *Aethina tu mida* (New Project)

Target: **Harrisia Cactus Mealybug**
Title: Pre-release quarantine evaluations of natural enemies for the biological control of harrisia cactus mealybug
Cooperator: Alejandro Segarra, University of Puerto Rico, Mayaguez
Funding: $48,950 ($28,950 ER / $20,000 CPHST) – BC Line
ADODR: Leyinska Wiscovitch (Matt Ciomperlik CPHST Cooperator)
Objective: Pre-release quarantine evaluations of natural enemies for biological control of the harrisia cactus mealybug, including determining the biology, life history, host range and potentially adverse environmental impacts of the imported natural enemies *Diadiplosis coccididvora* (Diptera: Cecidomyiidae) and *Leptomastidae sp.* (Hymenoptera: Encyrtidae).

Target: **Grasshopper**
Title: Development of a myco–acridacide, myco-insecticide and bacteria derived toxin as biorational control options for grasshoppers and Mormon crickets on rangeland
Cooperator: Stefan T. Jaronski, ARS-NPARL Sidney, MT
Funding: $7,000 – Grasshopper Line
ADODR: Nelson Foster
Objective: Conduct field cage trials to evaluate several strains of insect pathogenic fungi against Mormon crickets.

Target: **Imported Fire Ant**
Title: Biocontrol of the imported fire ant
Cooperator: Jason Byrd, FL DOACS Division of Plant Industries
Funding: $287,500 ($145,000 ER / $142,500 CPHST) – BC Line (Region) and IFA Line (CPHST)
ADODR: Ron Weeks, ER (Anne-Marie Callcott CPHST Cooperator)
Objective: To mass-rear and release permitted phorid fly species throughout IFA-infested states.

Target: **Imported Fire Ant**
Title: Support for IFA-phorid fly rearing program
Cooperator: Sanford Porter, ARS Gainesville, FL
Funding: $24,000 – IFA Line
ADODR: Anne-Marie Callcott
Objective: To perfect rearing techniques and for technical support when transitioning new species and biotypes of flies to FL-DPI for massing rearing: specifically transferring rearing and release techniques to DPI for *P. cultellatus* in FY10.

Target: ***Megacopta cribraria* Stink Bug**
Title: Evaluation of pest potential and development of management strategies for the invasive plataspid stinkbug, *Megacopta cribraria*, in Georgia.
Cooperator: Tracy Jenkins, University of Georgia
Funding: $10,000 – BC Line (CPHST contribution to a broader CA initiated by the ER)
ADODR: Bill Kauffman, ER SPHD-GA (Ken Bloem CPHST Cooperator)
Objective: To develop a database of DNA sequence fingerprints to help determine the geographic origin of this invasive pest, which would help focus the search for natural enemies should a classical biological control program be initiated.

Target: ***Sirex noctilio***
Title: *Amylostereum, Deladenus siricidicola*, and *Sirex noctilio* in North America
Cooperator: Dr. Ann E. Hajek, Entomology Department, Cornell University, Ithaca, NY
Funding: $71,254 – *Sirex* Program
ADODR: David W. Williams
Objective: To conduct studies on native species of *Sirex* and their associated fungi and nematodes and on the interaction between the Australian "Kamona strain" of *D. siricidicola* (nematode) and *A. areolatum* fungus strains already present in North America

Target: ***Sirex noctilio***
Title: Fungal symbionts and parasitic nematodes associated with *Sirex* species in North America and Europe
Cooperator: Dr. Ann E. Hajek, Entomology Department, Cornell University, Ithaca, NY
Funding: $97,520 – Sirex Program
ADODR: David W. Williams
Objective: To identify the species and strains of Amylostereum and strains of *Beddingia siricidicola* associated with *S. noctilio* and *S. juvencus* in Europe; to investigate the native North American parasitic nematodes and fungal strains associated with the North American native woodwasp, *Sirex nitidus*, that principally attacks spruce; to explore the potential non-target effects of use of the Kamona strain of *B. siricidicola* in North America.

Target: **Winter Moth**
Title: Mass-rearing and release of parasitoids versus winter moth in New England
Cooperator: Joe Elkinton, University of Massachusetts
Funding: $25,000 – BC Line (CPHST contribution to a broader CA initiated by the ER)
ADODR: Patricia Douglass, ER SPHD-MA (Ken Bloem CPHST Cooperator)
Objective: Release parasitoids for the first time in Rhode Island at Martha's Vineyard. Specific questions to be answered at this release site: (1) Should flies be released as soon as they emerge and mate or be held for an additional two weeks until females begin to lay eggs? (2) Should flies be held in growth chambers until they are ready to be released or should they be acclimatized to field conditions in outdoor holding cages?

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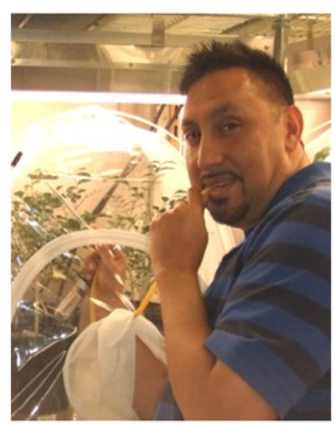

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Special thanks!

Leeda Wood will be retiring on September 30, 2011. She spent her entire 25-year USDA career working in the area of Biological Control at the Mission Lab on Moore Air Base, beginning in 1985 as a Biological Science Technician (Insects) and then from 1987-2005 as Entomologist. Some of the projects and activities she led or was associated with include: European corn borer, Colorado potato beetle, Russian wheat aphid, brown citrus aphid, boll weevil, silverleaf whitefly, purple loosestrife, glassy-winged sharpshooter, giant salvinia, *Arundo donax, and* Asian citrus psyllid. She is the Quarantine Officer for the Mission Lab's High Security Arthropod Containment Facility, and, since 2005, she has served as the laboratory Quality Manager. In addition to being a productive, enthusiastic member of the APHIS-PPQ Biological Control community, Leeda has been responsible for editing and formatting the CPHST BC Annual Report for the past two years.

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